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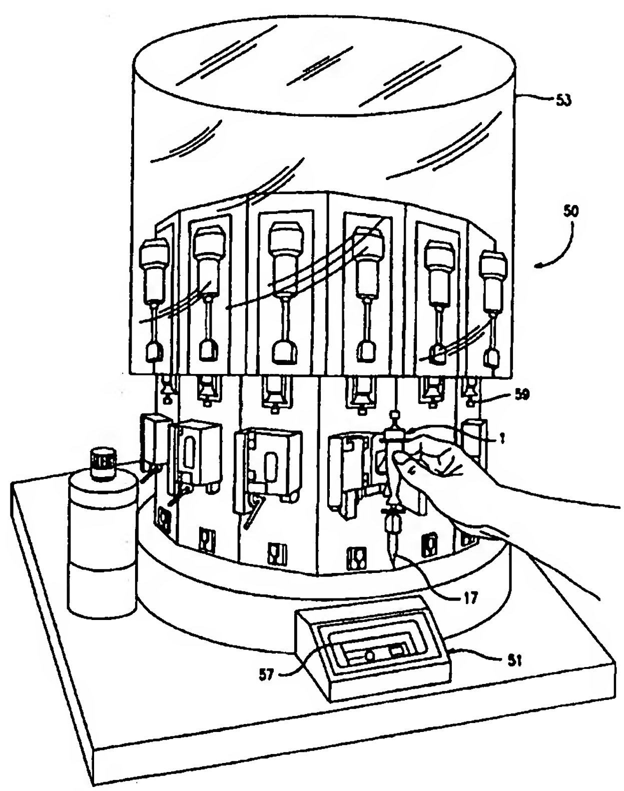
(54) Title: AUTOMATED NUCLEIC ACID EXTRACTION FROM SAMPLES

(57) Abstract

A closed sample processing system where wash fluids are pumped from closed containers, with any air that is allowed into the containers being filtered. Air that is pumped into the sample processing system, such as for drying solid phase, is filtered. Tubing from wash fluid containers or the air drier, allow fluid flow in one direction only, such that there can be no passage of nucleic acid sample (e.g. as an aerosol) into the tubing. The parts of the sample processing system that would be in contact with nucleic acid (and thus potentially act as sources for contamination) are the disposable device and the waste tubing. However, the disposable device is completely removable from the sample processor and can be disposed of after use, and the waste tubing allows fluid flow in only one direction, to waste, and thus will not act as a source of nucleic acid contamination.



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AUTOMATED NUCLEIC ACID EXTRACTION FROM SAMPLES

BACKGROUND OF THE INVENTION

The present invention relates to the isolation of a biological material, for example nucleic acid, from a basic material containing said biological material.

An example of a method for the isolation of nucleic acid is known from US Patent 5,234,809 to Boom et al., the subject matter of which is incorporated herein by reference. In this method, the basic material, a chaotropic fluid and silica particles are mixed, with the result that the silica particles are then separated from the fluid and treated with a buffered eluant, in which the nucleic acid is dissolved off the particles. With this method, HIV tests, for example, can be prepared by isolating the nucleic acid from the basic material, which can be blood or blood products such as serum or plasma.

More particularly, in the above-mentioned process, known as the Boom method, a sample is added to a lysis buffer containing guanidine thiocyanate (GuSCN) and a surfactant. Any viral particles and cells are thereby dissociated. Rnases and Dnases associated with the sample are inactivated. Nucleic acid is isolated by adding silicon dioxide particles to the sample lysis buffer cocktail. These particles

act as a solid phase to which the nucleic acid is bound, and are washed several times.

Unfortunately, the washing steps in the manual Boom method are very labor intensive and time consuming. To perform a washing step, the silica is centrifuged to form a pellet and the supernatant aspirated. The pellet must be then resuspended in a washing solution and the process repeated. The washing step is repeated five times, two times with wash buffer, twice with ethanol and once with acetone. After the pellet is sufficiently washed, it is then dried. Once dry, the nucleic acid is eluted from the silica using nuclease-free water as the eluant. The eluate contains nucleic acid, both DNA and RNA, from the starting sample. The nucleic acid in the eluate is concentrated and purified and is suitable for nucleic acid based diagnostics, e.g., direct probes and amplification based assays.

A very significant problem in automating the manual Boom method is the problem of cross-contamination. Because the steps following nucleic acid extraction steps, are often steps in an amplification method, even minute quantities of nucleic acid impurities due, for example, to cross-contamination, can be subsequently amplified so as to give erroneous results.

SUMMARY OF THE INVENTION

In accordance with the present invention, an automated apparatus, hereinafter called the "sample processor", is provided to perform automated nucleic acid extraction. The sample processor can be used in conjunction with a nucleic acid release kit, an isolation kit, and a disposable filter (called the "device" or the "disposable device" hereinafter). This combination provides a closed environment for the extraction of nucleic acid to take place. When a sample is placed in the lysis buffer, the nucleic acid is stabilized. The release kit is kept separate so samples can be lysed as shortly after collection as possible. Samples can be frozen for later processing with no degradation of the nucleic acid.

If a System Control, homologous internal control or heterologous controls is being used it would be added immediately prior to adding silicon dioxide particles to the lysis cocktail. Homologous control contains the same target sequence as the wild type target. They are distinguishable from the target by either size or the presence of internal sequences. Homologous controls are amplified with the same primers as the wild type target Heterologous control does not contain the same sequences as the target and requires a different set of primers to amplify. Nucleic acid and control bind to the silicon dioxide

particles. A system control is a segment of nucleic acid that acts as an internal control to verify that all the steps (extraction, amplification and detection) have been performed correctly. The control is extracted along with the RNA and DNA present in the sample. In the presence of the correct primers the control will co-amplify with the target. When used with the correct probes the system control can be detected along with the target.

The disposable device is characterized by a container for holding a mixture of the basic material, a chaotropic fluid and a solid phase which binds the biological material; means for separating the solid phase with the biological material from the fluid bound thereto; and means for connecting the container to an inlet and outlet for washing fluid for washing the biological material bound to the solid phase, to an inlet for an eluant fluid, and to an eluate reservoir for collection of the eluant with the nucleic acid and control.

If the solid phase which binds the biological material includes particle material, then it is advantageous if the means for separating the solid phase from the fluid are provided with a filter for allowing passage of the fluid and retaining the particle material.

The purpose of the solid phase is to fix or localize the nucleic acid so that debris and contaminants can be washed away. For this invention to work, the solid phase does not have to be in suspension with the lysis buffer and sample. The solid phase could be incorporated into the filter membrane itself. The solid phase could have various form factors, such as spun silica fibers filling the chamber in the disposable device, a sintered pellet of silica, a honeycomb structure of silica-based ceramic, etc.

The means for connecting the container to the inlet or outlet for eluant fluid and washing fluid and/or to the eluate reservoir are preferably provided with a shut-off element, which can be provided with, for example, a septum and an outlet channel for allowing through fluid from the separating means to the outlet, and also a hollow needle element connecting to the separating means, for piercing the septum in order to connect the separating means to the eluate reservoir. Such a shut-off element is simple, user-friendly and reliable in operation.

In a preferred embodiment of the disposable device, the container is provided with two sections lying one above the other and separated by a constriction, and is also provided with a supply element which can be connected to the inlet and is

movable between a position above the constriction and a position connecting in a close fit to the constriction.

In this embodiment both compressed gas for discharging the sample fluid from the container and the washing fluid and the eluant fluid can be fed into the container by one and the same supply element. During the infeed of gas the supply element will be in the position above the constriction, following which the supply element is moved to the position connecting suitably to the constriction, so that only the bottom part of the container, containing the solid phase with the biological material bound thereto, need be flushed, with the result that less washing fluid is needed and there is less of a risk of contamination occurring.

In an alternative embodiment the shut-off element is a valve element designed with at least one non-return valve between inlet and container.

In a more advanced development thereof, the valve element is provided with three non-return valves: the first-mentioned non-return valve in the connection to a compressed gas supply, a second non-return valve between the container and the separating means, and a third non-return valve in a connection between the separating means and the inlet for the washing fluid and the eluant. Compared with the embodiment with

one valve, this embodiment has the advantage that the washing fluid is introduced directly into the separating means, and the container therefore does not need to be washed, with the result that only a small amount of washing fluid is needed.

The invention will be explained below with reference to the drawings, which show a number of exemplary embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a vertical longitudinal section of a first embodiment of the disposable device which can be removed from the sample processor;

Fig. 2 is a section corresponding to Fig. 1, in which the device according to the invention is placed in a control apparatus ("sample processor");

Fig. 3 shows on a larger scale the detail III from Fig. 2, with the supply element in the bottom position;

Fig. 4 is a vertical section of an alternative embodiment of a part of the device according to Fig. 1, in which the shut-off element is in the washing position;

Fig. 5 is a section corresponding to Fig. 4, with the shut-off element in the elution position;

Fig. 6 shows on a larger scale the detail VI from Fig. 5;

Fig. 7 is a vertical longitudinal section of a second embodiment of the disposable device;

Fig. 8 is a vertical longitudinal section of a third embodiment of the disposable device;

Fig. 9 is a front view of the sample processor for holding a plurality of the disposable devices;

Fig. 10 is a close-up view of a disposable device being loaded into the sample processor;

Fig. 11 is a rear view of the sample processor showing the source of fluids used in the automated process;

Fig. 12 is a top view of the sample processor;

Fig. 13 is a front view of a second embodiment of the sample processor for holding a plurality of the disposable devices;

Fig. 14 is a vertical longitudinal section of one embodiment of a reagent bottle for use in the sample processor;

Fig. 15 is a vertical longitudinal section of a second embodiment of the reagent bottle for use in the sample processor;

Fig. 16 is a front view of the air dryer and sterilizer for the sample processor;

Fig. 17 is a vertical longitudinal section of one embodiment of the waste disposal system of the invention.

Fig. 18 is a sample processor functional block diagram;

Fig. 19 is a sample processor system block diagram;

Fig. 20 is a system controller block diagram; and

Fig. 21 is a sample processor device controller block diagram.

The drawings show exemplary embodiments of a sample processor and a disposable device for use in the isolation of a biological material, such as nucleic acid, from a basic material containing said biological material. The basic material can be, for example, blood, blood serum, urine, faeces, cell cultures and the like. The isolation of the biological material, in particular nucleic acid, is necessary for carrying out tests, such as, for example, an HIV test.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

First will be described the detachable/disposable element ("device") of the apparatus (the "sample processor") followed by a description of the sample processor itself. The device is made detachable/disposable so that it can be used as the point for isolating the nucleic acid, and can then be separated from the sample processor for removal of the nucleic acid therein. The detachable device can then

be disposed of (preferably) or carefully washed for further use. In this way, problems of contamination from one nucleic acid isolation to the next, can be avoided.

The disposable device shown in Figs 1 and 2 comprises a container 1 for holding a mixture of the basic material, a chaotropic substance and a solid phase which binds the nucleic acid, in this exemplary embodiment silica particles. What is meant by chaotropic substance is any substance which is capable of altering the secondary, tertiary and/or quaternary structure of proteins and nucleic acid, but leaves at least the primary structure intact. Examples thereof are guanidine, (iso)thiocyanate and guanidine hydrochloride. In this exemplary embodiment the container 1 belongs specifically to the device and the sample to be examined must be placed in the container by pipetting. The container is then sealed with a cover 2. The cover 2 is designed with an inlet connection 3, for connection of the container 1 to an inlet for compressed air, washing fluid and eluant fluid. These inlets for fluids form part of the sample processor, parts of which are shown in Fig. 2 and in which the device according to the invention can be placed for carrying out the isolation of the nucleic acid. Fig. 2 shows, for example, a connecting ring 4 for suspending the device in the apparatus, for

which purpose the container 1 is designed with a circular flange 5. Fig. 2 also shows a connecting element 6 for the inlets for the fluids, which connecting element can be connected to the inlet connection 3 of the cover 2.

The container 1 forms the top element of the device, which is connected at the bottom end to a bottom element 7. This cylindrical bottom element 7 comprises on the periphery an outlet connection 8 for connecting the device to an outlet for sample fluid and washing fluid, which forms part of the apparatus and is indicated by 9. Clamped between the top end of the bottom element 7 and the container 1 is membrane 10, which serves as a filter and on which the silica particles with nucleic acid adsorbed thereon can settle. A channel 11 connects to the space below the membrane 10. The channel 11, which forms the passage for a needle 12, comes out in a shut-off element 13, which in this case is provided with a septum 20. The shut-off element 13, made from, for example, a silicone material, is provided with an outlet channel 14 with a top part lying in line with the channel 11 and a bottom part running radially towards the periphery. At the periphery of the shut-off element 13 the outlet channel 14 opens out into an annular peripheral channel 15 which can be placed in communication with the outlet connection 8 in the

bottom element 7. In the position of the shut-off element 13 shown in Fig. 2 sample fluid and washing fluid can be conveyed out of the container 1, by way of the membrane 10 and the channels 11 and 14 to the outlet 9. A removable sealing plate 16 ensures that the outlet connection 8 is sealed before the device is used. The shut-off element could also be placed initially in a closed position and pushed to a discharge position only when the sample fluid is to be discharged.

The disposable device also comprises an eluate reservoir 17 for the collection of an eluant supplied from the inlet connection 3, and containing the nucleic acid dissolved off the silica particles. The eluate reservoir can be a standard cup with a capacity of, for example, 0.5 ml, which is shut off by a septum 18 of silicone material. The eluate reservoir 17 can be placed in a positioning element 19 of the apparatus, and with this positioning element 19 eluate reservoir 17 and shut-off element 13 can be pushed up relative to the bottom element 7 with the needle 12, in such a way that the needle cuts open in a sealed-off manner the septum 20 in line with the top part of the outlet channel 14 in the shut-off element 13 and the septum 18 of the eluate reservoir 17, following which eluate supplied can pass into the eluate reservoir 17 without the risk of leakages. A vent

channel 21 together with a vent groove in the periphery of the needle 12 (see Fig. 6) ensure that air can escape from the eluate reservoir 17 for the admission of the eluant fluid. The vent channel 21 in the shut-off element 13 can also be combined with the discharge channel 14, while a second needle can also be disposed in the shut-off element for the venting.

It can also be seen in Figs 1, 2 and 3 that a hollow cylindrical-shaped inlet element 23 is formed in line with the inlet connection 3 of the cover 2, which inlet elements projects until it is deep inside the container 1 and is movable in the container 1 due to the construction of the cover with flexible ridges 30. The container 1 is formed in two parts, namely a top part 25 of large volume tapering downwards toward a constriction 24, and a bottom part 26 of small volume and flaring out slightly downwards from the constriction 24, and connecting to the membrane 10. The inlet element 23 can be moved by means of the connecting element 6 of the apparatus between a top position shown in Figs. 1 and 2, in which the inlet element 23 opens out above the constriction 24 in the top part 25 of the container 1 and a bottom position, in which the feed element engages in a shut-off manner in the constriction 24 and therefore opens out in the bottom part 26 of the container 1. The inlet element

23 and/or the constriction 24 could be provided with snap means 27 for reliably maintaining the grip.

The device according to Figs. 1 - 3 works as follows:

First, the mixture of the basic material, the chaotropic substance and the silica particles is placed in the container 1, and the sealed device is then placed in the sample processor in the position shown in Fig. 2. Air is then pumped through the inlet connection 3 and the inlet element 23 into the container 1, in order to build up pressure in the container 1 for promoting the discharge of the sample fluid from it. After this discharge of the sample fluid, only the silica particles with adsorbed nucleic acid material remain behind on the membrane 10, together with residues of the sample fluid. The inlet element 23 is then moved to the bottom position in engagement with the constriction 24, following which a washing buffer (e.g. a mixture of salts), ethanol and acetone are fed in through the inlet element, in order to wash the silica particles and the cavities and passages of the device in question. Air can also be pumped through intermittently, in order to achieve an additional scraping effect. Finally, conditioned warm air is passed through. The next step is then to move up the positioning element 19, in order to move the eluate reservoir 17 and the shut-off element 13, so

that the septums 18 and 20 can be pierced by the needle 12, as a result of which the container 1 enters into communication with the eluate reservoir 17 by way of the filter channel 11. Finally, the eluant fluid, for example in the form of TE buffer, double distilled water or PCR buffer, is fed in through the inlet element 23. The eluant fluid is kept in contact with the silica particles for predetermined period, following which the eluant fluid is pumped further and passes by way of the membrane 10 and the channel 11 in a predetermined quantity, for example 100 μl, into the eluate reservoir 17. In this eluant fluid the nucleic acid is dissolved off the silica particles and is ready for testing. The shut-off element 13 and the eluate reservoir 17 are then moved down again, with the result that the needle 12 returns to the discharge position. Remaining eluant fluid is then pumped away to the outlet. When the needle 12 is withdrawn, the septum 18 closes automatically, so that a sealed reservoir 17 with the fluid to be examined is obtained. At this point the eluant reservoir alone can be removed for additional processing (nucleic acid detection and/or amplification) or the entire detachable/disposable device including the eluant reservoir can be removed from the sample processor.

Figs. 4 - 6 show a variant of the shut-off element 13 and the eluate reservoir 17, which in this

case are combined to a fixed unit and together can be moved between the washing or discharge position shown in Fig. 4 and the elution position shown in Fig. 5, moved upwards relative to the position shown in Fig. 4, in which elution position the needle 12 has pierced through the septum 20 between the outlet channel 14 and the eluate reservoir 17. Fig. 6 shows the above-mentioned vent groove 22 in the needle, which ensures that air can escape from the eluate reservoir 17 when the eluant fluid flows into the reservoir. The other structural elements are comparable to those in Figs. 1 - 3.

Fig. 7 shows an exemplary embodiment of the device according to the invention which is different in principle from the aforementioned embodiments. In this case the shut-off element is a valve element 28 on which a standard sample tube can be fitted as container 1. The valve element 28 is this exemplary embodiment contains three commercially available non-return valves: a non-return valve 29 in a connecting channel 30 between a compressed gas inlet and the container 1, a second non-return valve 31 between the container 1 and filter membrane 10 and channel 11 acting as separating means, and a third non-return valve 32 in a connecting channel 33 between the filter membrane 10 and the channel 11 in the inlet for the washing fluid and the eluant fluid.

Instead of a pierceable septum in the shut-off element, the valve element 28 is provided with a rotary valve 24 which is below the membrane 10 and the channel 11 and is for connecting the channel 11 as desired to the outlet connection 8 and the eluate reservoir 17 connected to the valve element 28.

This embodiment of the invention works as follows:

After the disposable device has been placed in the sample processor and the various inlets and outlets are connected, compressed air is supplied to the connecting channel 30, which compressed air passes by way of the non-return valve 29 into the container 1, and due to the pressure built up therein, the mixture of basic material, chaotropic substance and silica particles present therein is forced through the non-return valve 31 into the valve element 28, where the mixture is filtered and the silica particles remain behind on the membrane 10, and the fluid passes through the channel 11 and the rotary valve 24 into the outlet connection 8 and the connected outlet. The non-return valve 32 in this case remains closed, so that no fluid can pass into the connecting channel 33. Washing fluids are then introduced by way of the connecting channel 33 and the non-return valve 32 directly into the valve element 28 with the filter membrane 10, which can be washed with a relatively

small volume of washing fluid. The non-return valve 31 ensures that a seal is provided relative to the container 1. After the rotary valve 24 has rotated in order to produce the connection between the channel 11 and the eluate reservoir 17, eluate is fed through the connecting channel 33 and the non-return valve 32 to the valve element 28, and the eluant fluid with nucleic acid dissolved therein passes through the membrane 10 with silica particles and by way of the channel 11 into the eluate reservoir.

A further embodiment of the detachable/disposable device is illustrated in Fig. 8. This embodiment of the device shares many of the same structural elements as the embodiment illustrated in Fig 1. Below membrane 10, however, is disposed a rotary valve which is switched by the sample processor to allow fluid flow either to waste 8 or to eluate reservoir 17. Prior to loading the device into the sample processor, the eluate reservoir is held in place with needle 12 piercing septum 18 of the cap of the eluate reservoir. In this way, the additional step of moving upwardly the eluant reservoir to pierce the septum thereof as is performed during nucleic acid isolation in relation to the embodiment of Fig. 1, is not required.

The apparatus (the "sample processor") which utilizes the detachable/disposable device described above, will now be described. In one embodiment of

the sample processor illustrated in Figs. 9-12, a plurality of disposable devices are utilized. The sample processor in combination with the disposable device and reagents represents a closed system that minimizes contamination. This is important for assays that detect and/or amplify nucleic acid. Minimal contamination is achieved by ensuring one way flow for reagents and samples, allowing no sample in the primary flow path (waste lines only), keeping contamination within the disposable device, using filtered air for drying and reagent manipulation, and using closed reagent containers and collecting the eluate in a sealed container.

A computer controls a collection of pumps, valves and heaters to execute the method of extracting nucleic acid. The user can control the sample processor via a touch screen and a small display. The display provides control and status reporting. Sensors are provided to control and monitor proper execution of the process steps. Many protocols for extracting nucleic acid are possible with a sample processor of the present invention. For example, the sample processor can be utilized for performing procedures that optimize the time it takes to process samples of different starting volumes or sample types.

As can be seen in Fig. 9, a plurality of disposable devices 1 can be loaded into the sample processor 50. The eluate reservoir 17 of disposable device 1 is placed in a sensing element 19 of the sample processor to ensure correct assembly and installation of the disposable device. As can also be seen in Fig. 10, a door 55 is provided to be closed to hold the disposable device in place. The door can be held shut with a magnet, which activates a Hall effect sensor that indicates that the disposable device is properly loaded in place. Also, in order for the disposable device to be properly in place, inlet connection 3 of the disposable device must be properly connected to Luer connection 59 of the sample processor. In addition, connection 65 of the disposable device must be properly fitted to connection 66 of the sample processor, which provides for a fluid flow path for waste. Element 62 in Fig. 10 is a lower Luer fitting ejector which helps in removal of the disposable device. By pressing the free end of ejector 62 to the left in Fig. 10, the ejector pivots around a central point thereof so that the forked end of ejector 62 adjacent connection 66 is moved to the right so as to help dislodge connection 65 of the disposable device. Element 60 in Fig. 10 is a valve actuator for actuating the lower valve (e.g. rotary valve 24 in Fig. 8) of the disposable device

for directing fluid flow to a waste container (disposed, for example, in a bottom area of the sample processor) or to eluate reservoir 17.

As can be seen in Fig. 11, containers 70 holding fluids for use in the nucleic acid extraction process are disposed at the rear of the sample processor. The sample processor can be rotated around its base 79 to allow easy access to fluid containers 70. Also, operator console 51 can be disposed at any convenient location on the sample processor, or alternatively a separate assembly such as a personal computer connected to the sample processor, is envisioned. As an example, bottle 1 in Fig. 11 could contain wash buffer, bottle 2 could contain ethanol (e.g. 70% ethanol diluted in water), bottle 3 could contain acetone, and bottle 4 could contain the elution buffer.

Fig. 12 is a top view of the sample processor showing, among other things, fluid containers 70, doors 55 at various locations where the disposable devices are to be held, and operator console 51. The circular arrangement of the disposable devices in the sample processor is preferred and has a number of benefits. With a circular cross section, the sample processor can be made rotatable, as described above. Also, the tubing required to make a fluid connection between each fluid container and each disposable

device station, is much less in comparison to an apparatus having a linear arrangement of disposable devices.

Fig. 13 illustrates an alternate embodiment where the disposable devices are held in a linear array rather than in a circle as in Figs. 9-12. An advantage of this embodiment is that it can more easily be expanded to allow for more stations for holding disposable devices.

Fig. 14 is an illustration of a fluid container ("reagent bottle") for use in the sample processor. Reagent bottle 120 has attached thereto a cap 112. Tubing 151 is attached to cap 112 via quick release fitting 92 and makes a fluid connection to internal tubing 170 via gasket 100 and quick release fitting 88. Disposed at the bottom of the reagent bottle is a sinker 132 with a sinker adapter 145 attached thereto for connecting to internal tubing 170. Within sinker 132 is a filter 157 held in place by retaining ring 167 for blocking passage of impurities into flow tubing 170. As liquid in the reagent bottle flows out during sample processing, filtered air is allowed to pass into the bottle via syringe filter 142, luer adapter 133, and check valve 127 (held by check valve holder 111).

All the reagent bottles need not be the same size. For example, larger bottles can be used for

alcohol, acetone and wash buffer, and a smaller bottle (such as that illustrated in Fig. 15) could be utilized for elution buffer, due to the comparatively lower volumes of elution buffer that are used during sample processing. As can be seen in Fig. 15, a smaller sinker is disposed within the reagent bottle, such that no sinker adapter is used as in Fig. 14. Also, though the external dimensions of the cap are similar to the cap for the large reagent bottle, the cap has a narrow bottom portion 169 for fitting to the smaller diameter neck of the smaller bottle. Also, air passage 178 is disposed to provide an air passageway from check valve 127 to bottle 120.

Fig. 16 is an illustration of the inlet air dryer and sterilizer for connecting to the multi-port (e.g. 6-way or 8-way) valve (illustrated in Fig. 18). It is desirable that the air used during the nucleic acid extraction is dry and sterile. To achieve this end, a drying tube 200 (e.g. from Hammond Drierite/26930) is provided to dry air pumped in from ambient. Also provided is a filter for filtering out air impurities, preferably a filter made of polytetrafluoroethylene (e.g. 25mm Acrodisc filter from Gelman/4219). The filter is connected to the air dryer via vacuum tubing (e.g. Tygon tubing from Norton/AAC00020) and a polypropylene male luer lock. Also illustrated in Fig. 16 are female luer lock 205 and connector 207,

with polyethylene lined plastic tubing extending therebetween.

Fluids, both gas and liquid, flow through the disposable device during sample collection, and when eluate is not being collected in eluate reservoir 17 of the disposable device, the fluids otherwise flow to waste. As can be seen in Fig. 17, a waste container bottle 301 can be provided with a cap 302, both made from, for example, polypropylene. Waste tubing 303 is connected at one end to quick release fitting 315 (which connects to the main portion of the sample processor), and at the other end to in line quick release fitting 328. O-rings 311 are provided to create a fluid hermetic seal, and a panel mount quick release fitting 327 is provided to connect to internal waste tubing 303 (structural elements 315 and 316 corresponding to structural elements 328 and 327 mentioned above). Liquid waste is collected in waste bottle 301, whereas gaseous waste is passed to ambient via vacuum tubing 314, hose barb connector 310, and an activated carbon filtration device (e.g. Carbon Cap 150 by Whatman/6704-1500). It is important to filter well the gaseous waste, due to the use of a flammable gas like acetone in the nucleic acid extraction method. An acetone sensor could also be provided in the sample processor to monitor for any leaks.

Fig. 18 is a block diagram illustrating the closed nucleic acid extraction system. As can be seen at the top left of this diagram, the sample processor can optionally include a separate computer including a keyboard, barcode wand (the disposable devices might optionally include barcode labels), modem, printer, etc. An input and display (whether part of the separate PC system or via a touchscreen as illustrated in the figures) allows for a user interface with the system controller. Door sensors communicate to the system controller that the disposable device(s) are properly positioned in the sample controller. A six-way valve controller controls the 6-way valve which controls the flow of air and reagents to the disposable device station (the valve need not be a six-way valve, but could be 8-way or more).

A pump is controlled by a device controller for pumping air or reagents through the system. Also in communication with the device controller are the prime valve (used in priming the system prior to actual extraction), an eluate collection actuator and device sensor, and an eluate vial detector. The block diagram of Fig. 18 is only one embodiment of the present invention and is an illustration of a sample processor with two disposable device stations. Of course higher numbers of stations would be possible.

Before performing the nucleic acid extraction, a wash cycle is performed with each reagent to prime the system. For example, a predetermined amount of each reagent (e.g. 300 microliters) is displaced into the line that goes to the disposable device. Air is pumped to follow the predetermined amount of each reagent so as to pass the reagent through the system as a fluid "pulse". After priming the system, the nucleic acid extraction is performed. The sample fluid containing the nucleic acid and solid phase is pushed out of the disposable device past the disposable device filter, such that solid phase with nucleic acid (DNA or RNA) bound thereto is caught on the disposable device filter. Of course, as mentioned previously, the disposable device filter could be the solid phase itself, such that nucleic acid binds directly to the filter.

Then, at least one, and preferably more than one, wash fluid is pumped from wash reagent source(s) to wash the solid phase and nucleic acid. For example, one or more pulses of wash buffer (predetermined amounts of wash buffer containing a chaotropic substance followed by filtered air) are passed over the disposable device filter, followed by one or more pulses of alcohol (e.g. ethanol), followed by one or more pulses of acetone -- to wash away impurities from the solid phase and nucleic acid. Then the solid

phase/nucleic acid is dried with air pumped into the system via the air drier/sterilizer (Fig. 16) to remove traces of acetone. The upper luer fitting for connecting to the inlet of the disposable device can be fitted with a heater to help dry the acetone during this step. Following washing and drying of the solid phase, the nucleic acid is removed from the silica with an elution buffer, with a quantity of eluted nucleic acid being caught in the eluate reservoir of the disposable device.

Next, in relation to Figs. 19-21, is a description of the sample processor control system, system controller firmware and device controller firmware. The control of the sample processor can be implemented utilizing memory control as disclosed in U.S. patent application 08/404,121 filed March 14, 1995 (Edward B. Ramey), the subject matter of which is incorporated herein by reference.

I. Sample Processor Control System Architecture

1. Overview

The sample processor controls the flow of buffers and reagents through a biological sample in order to elute and collect amplifiable nucleic acid. Solenoid valves route the fluid materials through tubing lines which are pressurized by syringe pumps. Elution is performed in a disposable device which holds solid phase and a filter. This device can be heated to the

optimum temperature for elution. Sensors monitor placement of the device and the position of valves within the device.

The sample processor comprises ten such devices, each with an associated pump. For each set of two devices, a microcontroller module termed a device controller manages the syringe pumps, valves, heaters and other device-specific functions. Another microcontroller termed a system controller manages the user interface, initialization and other system-wide functions. This modularity enables system design flexibility and serviceability.

The system controller sends commands to device controllers through an internal network. A device controller can also send unsolicited status messages to the system controller. The system controller is assigned the master address on the network. Microcontroller hardware handles message routing and arbitration.

When the sample processor is powered on, all microcontrollers perform self tests on their local code and data memory and initialize attached devices. The system controller determines which device controllers are ready to run by sending queries to them and listening for a response. If all device controllers are ready, the system controller enters a

system ready state, awaiting a command from the operator.

2. Operator Interface

The system can be operated stand-alone or by commands sent to its host interface port. The operator interacts with the stand-alone instrument through an LCD character display with a touch-sensitive overlay. A message window on the display shows status items such as time to completion, warming up, standby, and temperature. The system can be configured to provide messages in English, German, French or a user-defined message set.

The host interface port is an RS-232 serial port which can accommodate a terminal, laboratory information system, personal computer, or modem. An expanded level of diagnostics is available from the serial port for long-term error logging and for teleservicing applications.

The system controller sends error messages to the status display and the host interface port whenever it detects a problem. The time of occurrence is appended to error messages going to the host interface port. The system controller also maintains in its memory an error history log (up to a limit of 150 errors). This log may be sent to the interface port on command, and

it may be viewed on the display through a service menu command.

3. System Controller Functions

The system controller sends commands to the device controllers and monitors their status. It accepts commands from and sends status messages to a host interface port. In addition, the system controller is assigned other system-level functions.

The system controller sends commands to a device controller to select the position of the multiport valve which is connected to one of the syringe pumps. This selects the working fluid for the entire system.

The system controller sends commands to a dedicated motor driver which is connected to the internal network. This driver actuates the "airpen" mechanisms which create a fluid path through the device filter. The controller reads sensors which monitor the position of these mechanisms and verify proper operation.

Sensors monitor the instrument cover doors and the supply and waste reservoirs (wash buffer, alcohol, acetone, elution buffer, auxiliary reagent, waste reservoir, and auxiliary reservoir). If the system controller detects an empty supply bottle or full waste reservoir, it sends an error message to the status display and to the host computer. If a door

sensor shows a door to be open while the instrument is running, the system controller sends a status message and lowers the setpoint temperature for the heaters to a safe contact level.

The system controller manages one heater for the reagent block. The temperature of the reagent block and the air are maintained at a setpoint of 56°C. The relative humidity of the air is also monitored. If the temperature goes out of range, the controller sends an error message to the status display and to the host computer. In order to allow time for heaters to reach nominal temperature, the system controller waits a specific time after the control process begins before it announces temperature out-of-range errors.

Two system voltages are monitored. If a voltage is out of range, the system controller sends an error message to the status display and to the host computer.

4. Macros

A sequence of device controller commands can be defined and assigned a name. Such a sequence is termed a macro. The sequence can then be invoked by sending a simple macro execution command. Macros may invoke other macros to a nesting of six levels deep.

Macros may use variable parameters. If an error occurs during execution of a macro, the macro

terminates, and any macros which follow in the sequence are canceled.

Macros are defined for common operations which the system performs, such as wash, rinse, displace and clean. This enables the system controller to operate within a high-level command structure which conserves memory and limits traffic on the internal network. Macros are maintained in nonvolatile memory in the device controllers. System operations are associated with macro names. In response to a command from the user interface, the system controller may simply send a run-macro command to the device controllers.

5. System Controller Memory

The system controller includes a block of battery-backed memory which stores system configuration information. It also contains a clock-calendar circuit which supplies data when an error report is time-stamped.

The system controller memory includes its own operating program code as well as a copy of the device controller operating program. This memory can be reprogrammed by sending appropriate commands and data to the host interface port. Similarly, the system controller can reprogram the memory on the device controllers by sending commands and data on the internal network. In this way system code revisions

can be made by connecting a personal computer or a modem to the host interface port.

6. Device Controller Functions

Each device controller sends commands to operate two syringe pumps through an RS-232 serial port. One pump connects to the RS-232 pump and routes commands for the second pump to a pump command bus. While the pump is active, pressure sensors check for excess pressure levels in the tubing; high levels can indicate obstructions in the tubing or device.

Devices are heated in order to provide proper temperature for the nucleic acid isolation process. The device controller provides current to the heater and monitors the temperature of the device for closed loop control.

The device controller supplies current to actuate solenoid valves which select the fluid path through the device for priming, washing or reagent dispensing. It also drives a motor attached to each device which moves a valve for eluate flow to a collection cup.

Position sensors in each device verify proper operation of the airpen which controls fluid flow within the device and the eluate removal valve. The device controller also reads a sensor which signals that a device or a shunt is properly inserted in the instrument.

7. Process Structure

The sample processor executes predefined methods which consist of a number of phases. The user selects a method to perform by pressing buttons on the user interface panel. The system controller then sends commands to all device controllers to start each phase. The system controller maintains coordination of device controllers when necessary for use of shared mechanisms, such as the airpen actuator and multiport valve.

Process phases follow one of two possible sequences, depending upon whether an extraction process or a cleaning process is selected. These sequences are:

LOAD

SAMPLE	CLEAN
WASH	TOP
ETOH	
ACETONE	
WASH2	
WASH3	
DRY	
ELUTE	
WASH4	
COOL	
UNLOAD	

II. System Controller Firmware Specification

1. Introduction

The following is a functional description of the system controller firmware. The system controller monitors and/or controls the following:

communications to device controllers
program loading for device controllers
device controller initialization
macro commands and response monitoring for device controllers
error reporting and logging
system configuration memory
real-time clock
operator interface panel (touch pad, display and beeper)
reservoir level sensors (supply and waste)
door unlock solenoid (which can be several solenoid locks wired in parallel)
send commands to satellite I/O module to control airpen shuttle
one door sensor (which can be several door sensors wired in together)
one reagent block heater
one air humidity sensor
24 volt supply
5 volt supply
communications to host computer or modem

(not required for operation)

Sensors monitor the instrument cover doors and the supply and waste reservoirs (wash buffer, alcohol, acetone, elution buffer, auxiliary reagent, waste reservoir, and auxiliary reservoir).

2. Physical Communications Interfaces

The system controller communicates with the device controllers through the IIC (inter-integrated circuit) control network. Effective data rate of this network is about 80 Kbps. A device controller can send unsolicited messages to the system controller. The system controller is assigned the master address on the network; network hardware handles message routing.

The system controller communicates with a host computer through an RS-232 interface. Serial port parameters may be user-configured; default settings are 19200 bps, even parity, 7 data bits, 1 stop it. The interface includes a DSR line for hardware flow control.

3. Command and Error Messages

The system controller sends error messages to the status display and the host interface port whenever it detects a problem. The time and date of occurrence are appended to the error message. The system

controller maintains in its memory an error history log (up to a limit of 32 errors). This log may be sent to the interface port on command, and it may be viewed on the display through a service menu command.

An LED lamp mounted on the system controller provides a base level of status information. When power is applied to the controller and it detects no active faults, the LED will blink at about 2 pulses per second. If a memory fault is detected, the rate will decrease to about once every two seconds. If the LED is on continuously, the flash EPROM or the processor probably needs to be reloaded. If the LED does not turn on at all, a power supply fault is likely.

4. Power Up

On power up, the system controller program first tests its program memory, internal ram memory, and external ram memory. If an error is found, a message is sent to the host interface and to the status display. If these are OK, the device controllers on the internal network are queried for status. If necessary, the system controller will upload the program memory to device controllers across the network. If any device controller fails to initialize, the system controller sends an error message to the status display and host interface, and

if possible, the instrument will continue to operate in a partial capacity.

The system controller then sends the prompt '/' to the host interface and initializes the status display with the start-up screen. The host can query the current revision level of the program by sending the 'RV' command, and if desired, the host can upload a different system controller or device controller program file. While the controller program is running, a continuous program memory checksum test executes to verify that memory is intact. If the test fails, the system controller sends a program memory checksum error and enters a graceful shutdown sequence. In this mode, the controller continues to monitor the host interface and it will accept a program file for download.

If an error is detected in any of the variables which the system controller continuously monitors, including temperatures, humidity, voltages, access doors, reservoir levels and communication channels, the controller will immediately announce the error to the status display and the host interface.

III. Device Controller Firmware Specification

1. Introduction

The following is a functional description of the device controller firmware. The device controller monitors and/or controls the following:

communications to two syringe pumps

communications to one multiport valve

pressure sensors for two devices

priming solenoid valves for two devices

bypass solenoid valves for two devices

valve actuator for eluate removal shuttle

four device heaters

device-in-place sensors for two devices

limit switches for airpen and eluate removal shuttles

24 volt supply

5 volt supply

Sensors monitor the pressure in the tubing, device temperature and device presence for two devices. If the device controller detects an excessive pressure, it disables the relevant pump and sends an error message to the system controller. The system controller may send a message to query the status of an individual sensor, or a string of data containing all of the current sensor states can be sent upon request.

Device controller firmware consists of two major partitions which are largely independent. The partition in the lowest 16 kilobyte block of memory holds the bootup program and the macros. The remaining 48 kilobytes hold the operating program. Normally the bootup program performs a memory test of the EPROM and RAM after power-up, then it jumps to the operating program. If a problem is detected during the memory test, the bootup program remains in control and will allow the system controller to upload a copy of the device controller operating program into its EPROM.

2. Physical Communications Interfaces

The device controller communicates with the system controller through the IIC (inter-integrated circuit) control network. Effective data rate of this network is about 80 Kbps. A device controller can send unsolicited messages to the device controller. The device controller reads the setting of a DIP switch to determine its address on the network; the network hardware handles message routing.

The device controller communicates with two syringe pumps through an RS-232 (optional RS-485) serial interface. Serial port parameters may be user-configured; default settings are 9600 pbs, no parity, 8 data bits, 1 stop bit.

3. Command and Error Message Formats

The system controller sends error messages to the status display and the host interface port whenever it receives an error message from the device controller. The system controller time stamps each error and maintains in its nonvolatile memory an error history log.

An LED lamp mounted on the device controller provides a base level of status information. When power is applied to the controller and it detects no active faults, the LED will blink at about 2 pulses per second. If a memory fault is detected, the rate will decrease to about once every two seconds. If the LED is on continuously, the flash EPROM or the processor probably needs to be reloaded. If the LED does not turn on at all, a power supply fault is likely.

4. Power Up

On power up, the device controller program first tests its program memory, internal ram memory, and external ram memory. If an error is found, a message is sent to the system controller. If the memory test results are OK, the device controller waits for a query from the system controller to report its status. If the system controller does not receive the proper response to its query, it marks the device controller

"inactive", reports an error, and continues to run with the other active device controllers.

While the device controller program is running, a continuous program memory checksum test executes to verify that memory is intact. If the test fails, the device controller sends a program memory checksum error and enters a graceful shutdown sequence. In this mode, the controller continues to monitor the system network and will accept a program file for download from the network.

If an error is detected in any of the variables which the device controller continuously monitors, including temperatures, pressures, voltages, and pumps, the controller will announce the error to the system controller.

5. Diagnostics and Monitoring

All DC voltages provided to the controllers are continuously monitored. The voltages should be within +/- 5% of the nominal value. Temperatures and pressures are also continuously monitored if the setpoint is not 0.

The device controller continuously monitors the pressure in the tubing while fluid is moving. If pressure exceed a programmed threshold, the pump is stopped and an error message is sent.

IV. Principle of Operation

The process begins by lysing a sample. The appropriate amount of sample is placed in a tube containing lysis buffer (Release Kit). This must be done a short time after sample collection to prevent degradation of the sample by nucleases. Lysing a sample stabilizes the nucleic acid. Sample lysis cocktail can be processed further or frozen for later testing.

The following steps take place in a laminar flow hood. Before starting these steps make certain that the instrument is turned on. Add 100 ul of silicon dioxide to the sample lysis buffer cocktail, mix gently several times during a 10 minute incubation at room temperature. Open an individual disposable filter and stand it up in the holder provided within the packaging. Remove the cap and pipette the sample, silica and lysis buffer cocktail into the disposable filter. Tighten the cap securely, install the eluate collection cup and place in a rack for transport to the sample processor. Repeat these steps until as many as 10 disposable filters are prepared.

Move the prepared disposable filter from the laminar flow hood to the instrument. Ensure there are enough reagents present to complete the process. The instrument checks this but it is more convenient to check before starting. From the operator console

select the run option, choose the correct extraction protocol, open the guard and begin loading the disposable filters. When installing the disposable filter make certain that the waste fitting is securely connected and the door is closed. Then make the Luer connection to the top of the disposable filter. After loading all the disposable filters close the guard and select start.

Upon starting, the sample lysis silica cocktail is pumped through a disposable filter. Silica is captured and the lysis buffer passes through to waste. Once all the cocktail has been filtered the silica pellet on the filter is washed with the following: wash buffer, 70% EtOH, acetone and dried. At this point a silica pellet with the bound nucleic acid is on the filter. Flow is diverted from waste to the eluate collection cup. The nucleic acid is eluted using water as an eluant. After elution, air is used to force residue reagents to waste.

Open the guard and remove the disposable filters, placing them in a rack for transport to the laminar flow hood. Once in the hood the eluate collection cups can be removed and the rest of the disposable filter thrown out. At this point the eluate is ready for amplification or direct probe testing or it can be frozen for later testing.

At power up the computers, six of them, go through a self diagnostic and initialization setting all sensors and actuators to their prescribed states. The six computers are arranged with one supervisor and five slaves. The supervisor controls all slave function error reporting, display functions, keypad functions and all connections to external equipment.

There are several key subsystems: base, turntable, pump housing, dual device module, reagent housings, display pod, safety cover and waste container.

The base houses the power supply and distributes power. AC power ranging from 90 to 250 volts and 50 - 60 Hz is received by the power supply and rectified to 24VDC. An umbilical from the base connects to the display pod and another to the waste container. Rising from the center of the base is an axle that has a bearing attached. The turntable is attached to the bearing. Between the base and turntable is a cable management system. The design can support a plurality of cable management approaches. The first is a cable wrap-up technique using a cable guide to spiral wind the wiring and tubing to the waste connection around the axle. The other is a torsional approach where the wiring and tubing is located on the axis of rotation and only exposed to torsional forces.

Attached to the turntable are five dual device modules, lower reagent housing and the pump housing. The dual device module is where the disposable filter holder connects to the instrument. When properly installed the disposable must have its valve handle in the valve actuator slot; its waste connection to the instruments waste connection and the inlet connected to the instruments inlet connection. When properly installed, the door will close. If it is not properly installed, the door will not close. The presence of the disposable is sensed along with the connection of the inlets and the door closing. When these conditions are met the instrument reports that the disposable is correctly installed. The inlet and waste fittings have check valve in them to prevent fluids from flowing in the wrong direction. The inlet fitting is heated to facilitate the drying step which is to remove any residual acetone.

Lower reagent housing and upper housing have sensors to detect the presence of liquid. These are set to a height that insures there is always enough reagent available to complete a test. The caps for the reagents are designed to provide an as closed as possible environment. This is accomplished by using filters on the connection that allows air to fill the space vacated by the liquid as it is consumed. A filter is on the end of the line that is connected to

the suction side of the pump to prevent contaminants from entering the instrument. A self sealing quick disconnect fitting is used to make changing the reagents simple.

The pump housing is attached to the turntable. Within the pump housing are 10 pumps and respective pressure sensors, the upper reagent housing and a multiport valve that allow selection of the correct reagent. The pumps force liquid through the disposable filter holder at a preset speed and volume. Pressure sensors monitor the pressure to insure the filter has not ruptured or the pressure is not too high. The pressure sensor is also used to determine when the silica cake on the filter is wetted with elution buffer. An increase in pressure indicates that the silica and membrane are wetted. During sample displacement the pressure sensor is used in a feedback control scheme to optimize the time it takes to displace the sample. Measuring the pressure provides indications on obstructions or leaks in the fluidic path. Small leaks are identified by a sensor that detects acetone and alcohol vapors. It is very sensitive. Approximately 50 PPM, this would be a spill of a few drops within the closed space of the sample processor.

It is also envisioned as part of the present invention that the final step in the process described

herein is an amplification and/or nucleic acid detection step. Upon removal of the disposable device after eluate reservoir 17 contains the required eluate, an amplification of the nucleic acid can be performed, such as NASBA or PCR. Alternatively or in addition, final detection steps for detecting the nucleic acid can further be performed.

The invention is not restricted to the exemplary embodiments shown in the drawings and described above, which can be varied in various ways within the scope of the invention. For example, mention is always made above of the sample fluid being discharged by means of compressed air or another compressed gas, but it is, of course, also possible to force the sample fluid out of the container 1 by mechanical means. For example, a hollow plunger could be provided in the essentially cylindrical container, in which case a small channel in the plunger is initially shut off by a seal and through the downward movement thereof in the container, the fluid in the container is pressed out through the filter. In the bottom position, the seal can then be pierced, following which the washing fluids and finally the eluant fluid can be supplied through the channel connected to the inlet in the plunger. Alternatively, it is also possible to fit a second plunger in the channel in the hollow plunger, which second plunger can be moved up and down and,

after the sample fluid has been expelled, ensures that washing fluids are extracted from an inlet which is connected to the valve element by means of a non-return valve, and that said washing fluids and possibly also the eluant are then passed through the filter and the valve element. In the lowest position of the hollow plunger the plunger seals off the largest part of the container relative to the shut-off element, so that only a small volume needs to be washed, and a small quantity of washing fluid will therefore suffice.

It is also pointed out that, instead of the slidable shut-off element shown in Figs. 1 - 6, a rotary shut-off element can be used. This can be comparable to the rotary valve of Figs. 7 and 8, but it is also possible to form the passage channels as recesses on the periphery of the rotary valve. In one position a first recess provides the passage to the outlet, while this first recess in a second position provides the venting, and a second peripheral recess permits the passage of eluant fluid to the eluate reservoir. The needle elements for eluate passage and venting are formed at a position below the rotary valve in this embodiment.

As can be seen from the description above, the labor intensive steps involved in the manual method are eliminated. At the same time, the serious

problems of contamination of sample are eliminated due to the closed sample processing system (e.g. in comparison to a system that would suffer from cross-contamination, such as a microtitre plate system). In the novel closed system of the present invention, wash fluids are pumped from closed containers, with any air that is allowed into the containers being filtered. Air that is pumped into the sample processing system, such as for drying solid phase, is filtered. Tubing from wash fluid containers or the air drier, allow fluid flow in one direction only, such that there can be no passage of nucleic acid sample (e.g. as an aerosol) into the tubing. The parts of the sample processing system that would be in contact with nucleic acid (and thus potentially act as sources for contamination) are the disposable device and the waste tubing. However, the disposable device is completely removable from the sample processor and can be disposed of after use, and the waste tubing allows fluid flow in only one direction, to waste, and thus will not act as a source of nucleic acid contamination.

WE CLAIM:

1. An automated apparatus for extracting nucleic acid, comprising:

a computer controller for controlling said automated apparatus;

a housing for holding a sample containing nucleic acid;

a solid phase enclosed in said housing, said solid phase capable of binding to said nucleic acid;

at least one wash fluid container for holding a wash fluid for washing the solid phase with said nucleic acid bound thereto;

a first fluid conduit for placing said housing in fluid communication with said wash fluid container;

at least one elution fluid container for holding an elution fluid for removing said nucleic acid bound to said solid phase;

a second fluid conduit for placing said housing in fluid communication with said elution fluid container;

a waste container for collecting waste fluid from said housing;

a waste conduit for making a fluid connection between said housing and said waste container; and

an eluate reservoir for collecting elution fluid containing said nucleic acid from said housing.

2. An apparatus for automating the extraction of nucleic acid from a sample, comprising:

a controller device for controlling electrical and/or mechanical functions of said apparatus;

a housing separable from the apparatus and for holding a solid phase capable of binding nucleic acid;

at least one wash fluid container for holding a wash fluid for washing the solid phase with nucleic acid bound thereto within said housing;

at least one elution fluid container for holding an elution fluid for eluting nucleic acid bound to said solid phase;

at least one wash fluid conduit for placing said housing in fluid communication with each said at least one wash fluid container;

at least one elution fluid conduit for placing said housing in fluid communication with each said at least one elution fluid container;

a waste conduit for the passage of waste from said housing.

3. The apparatus of claim 2, further comprising an eluate reservoir connectable to said separable

housing for collecting elution fluid containing nucleic acid from said housing.

4. The apparatus of claim 3, wherein both said housing and said eluate reservoir are separable from the apparatus and from each other.

5. The apparatus of claim 2, wherein said housing contains a filter therein for holding solid phase within said housing, said housing being disposable and designed for single use for minimizing cross contamination between tests.

6. The apparatus of claim 4, wherein said housing comprises needle for piercing a septum of said eluate reservoir.

7. The apparatus of claim 2, further comprising an air pump for pumping air through said housing.

8. The apparatus of claim 6, wherein said needle comprises a vent groove for allowing air to pass out of said eluate reservoir when elution fluid flows thereinto.

9. The apparatus of claim 3, further comprising a valve in said housing for directing fluid to waste or to said elution reservoir.

10. The apparatus of claim 9, wherein said valve in said housing is a rotary valve.

11. The apparatus of claim 9, further comprising means for determining that said housing is properly positioned within the automated apparatus.

12. The apparatus of claim 11, further comprising an ejector mechanism for dislodging said housing from said apparatus after performance of a particular assay.

13. The apparatus of claim 12, wherein said ejector mechanism comprises a forked end and a pivot point for allowing the ejector mechanism to pivot around said pivot point for dislodging said housing.

14. A closed automated system for the extraction of nucleic acid from a sample, comprising:

a controller device for controlling electrical and/or mechanical functions of said apparatus;

a housing separable from the apparatus and for holding a solid phase capable of binding nucleic acid,

said housing having at least one inlet and at least one outlet;

a wash fluid container for holding a wash fluid for washing the solid phase with nucleic acid bound thereto within said housing, said wash fluid container having an outlet for passage of wash fluid to said housing, and an inlet for air, wherein proximate to said wash fluid container inlet is a filter for filtering out airborne and/or microbial contamination;

an elution fluid container for holding an elution fluid for eluting nucleic acid bound to said solid phase, said elution fluid container having an outlet for passage of elution fluid to said housing, and an inlet for air, proximate to said inlet being a filter for filtering out airborne and/or microbial contamination;

a wash fluid conduit for placing said housing in fluid communication with said wash fluid container;

an elution fluid conduit for placing said housing in fluid communication with said elution fluid container;

a waste conduit for the passage of waste from said housing.

15. The closed automated system of claim 14, further comprising a waste container in fluid communication with said waste conduit for collecting

waste from said housing, said waste container being equipped with means for preventing aerosols and vapors from being released to ambient.

16. The closed automated system of claim 15, wherein said housing forms a closed fluid circuit from said wash fluid and elution fluid containers to said waste container, and wherein nucleic acid contained within said closed system is restricted to said separable housing or points downstream therefrom.

17. The closed automated system of claim 16, wherein said separable housing comprises an eluate reservoir for collecting eluate from solid phase within said housing.

18. The closed automated system of claim 17, wherein said eluate reservoir is separable from said housing, and wherein said eluate reservoir, when connected to said housing, is closed off from ambient for minimizing nucleic acid contamination to ambient.

19. The closed automated system of claim 18, further comprising an air inlet with air filter, and an air conduit for allowing the passage of filtered air to said housing.

20. The closed automated system of claim 19, further comprising a heater for heating air passing from said air inlet to said housing.

21. An apparatus for automating the extraction of nucleic acid from a sample, comprising:

a controller for controlling the flow of reagents through said apparatus;

a housing having a filter and for holding solid phase therein;

a plurality of reagent containers in fluid communication with said housing;

at least one pump for pumping reagents through said apparatus;

at least one valve for controlling reagent flow through said housing;

a waste line in fluid communication with said housing for passing waste from said housing.

22. The apparatus of claim 21, wherein said controller comprises at least one device controller for controlling said at least one pump and said at least one valve.

23. The apparatus of claim 22, wherein said controller further comprises at least one system

controller for managing a user interface and initialization of the apparatus.

24. The apparatus of claim 23, wherein said system controller sends commands to said device controller and monitors the status of said device controller, and wherein said system controller accepts commands from and sends status messages to a host interface port.

25. The apparatus of claim 23, further comprising sensors for monitoring proper placement of said housing and for monitoring said reagent containers.

26. The apparatus of claim 25, further comprising a waste container in fluid communication with said waste line.

27. The apparatus of claim 26, further comprising a sensor for monitoring said waste container.

28. A method for extracting nucleic acid from a sample in a machine providing a closed environment, comprising:

providing a controller for controlling machine functions;

providing a housing within said machine, the housing having therein a sample containing a nucleic acid, the housing further including therein a solid phase for binding to said nucleic acid;

causing said sample to flow from said housing to a waste conduit, leaving said solid phase with said nucleic acid bound thereto in said housing; and

causing a wash fluid from a wash fluid source to flow through a fluid conduit to said housing to wash said solid phase and nucleic acid, said wash fluid passing through said housing to said waste conduit.

29. The method of claim 28, further comprising:

after washing said solid phase, causing an elution fluid from an elution fluid source to flow through a wash fluid conduit to said housing so as to come in contact with said solid phase and remove at least part of said nucleic acid from said solid phase; and

collecting at least part of said elution fluid with said nucleic acid therein as said elution fluid passes from said housing to an eluate reservoir.

30. An automated method for extracting nucleic acid from a sample comprising:

providing an apparatus having a controller for controlling electrical and/or mechanical functions of said apparatus, and at least one wash fluid container;

connecting a housing to said apparatus, said housing having therein a sample containing a nucleic acid and a solid phase for capable of binding to said nucleic acid, said housing and being connected to said apparatus so as to be in fluid communication with said at least one wash fluid container;

causing said sample to flow from said housing to a waste conduit, leaving said solid phase with at least part of said nucleic acid bound thereto in said housing; and

causing a wash fluid from said at least one wash fluid container to flow through a wash fluid conduit to said housing to wash said solid phase and nucleic acid, said wash fluid passing through said housing to said waste conduit.

31. The method of claim 30, further comprising removing said housing with said nucleic acid therein from said apparatus and amplifying and/or detecting said nucleic acid.

32. The method of claim 30, further comprising: providing at least one elution fluid container, and wherein when said housing is connected to said apparatus, said housing is placed in fluid communication with said elution fluid container; and

after said washing of said solid phase and nucleic acid, causing an elution fluid from said elution fluid container to flow through an elution fluid conduit to said housing so as to come in contact with said solid phase and remove at least part of said nucleic acid from said solid phase so as to form an eluate comprising nucleic acid.

33. The method of claim 33, further comprising:

providing an elution reservoir connectable and detachable to said housing; and

collecting at least part of said eluate with nucleic acid therein in said elution reservoir.

34. The method of claim 33, further comprising detaching said housing from said apparatus and detaching said elution reservoir with eluate therein from said housing.

35. The method of claim 34, further comprising disposing of said housing and amplifying and/or detecting nucleic acid in said eluate.

36. A method for extracting nucleic acid from a sample, comprising:

providing the automated system of claim 14;

providing a wash buffer containing a chaotropic substance in said wash fluid container, providing an elution buffer in said elution fluid container, and providing a filter and solid phase in said housing;

passing a predetermined amount of said wash buffer over said solid phase and filter to wash said solid phase; and

passing a predetermined amount of said elution buffer over said solid phase to elute said nucleic acid off of said solid phase so as to form an eluate containing nucleic acid.

37. The method of claim 36, further comprising:

providing containers for each of alcohol and acetone and pumping predetermined amounts of alcohol and acetone over said solid phase in said housing before said elution step.

38. The method of claim 37, further comprising:

providing an air pump system and pumping air through said housing to remove traces of acetone therein prior to said elution step.

39. The method of claim 38, wherein said pumped air is heated.

40. The method of claim 38, further comprising:

providing an elution reservoir connectable and detachable to said housing; and

in said elution step, collecting at least some of said eluate in said elution reservoir.

41. The method of claim 40, further comprising amplifying said nucleic acid in said eluate.

42. The apparatus of claim 41, further comprising a valve actuator for actuating said rotary valve for directing said fluid to waste or to said elution reservoir.

43. The device according to claim 2, further comprising an inlet element which can be connected to the inlet and is movable between a top position for discharging sample fluid and a bottom position for passing through washing fluid and eluant fluid.

44. The device according to claim 43, in which the container is provided with two sections lying one above the other and separated by a constriction, and the inlet element is movable between a position above

the constriction and a position connecting in a close fit to the constriction.

45. The device according to claim 44, in which the inlet element is in the form of a hollow pin having snap elements at the end for engagement with the constriction.

46. The device according to claim 44, in which the inlet element is suspended from a flexible cover of the container, which permits the movement of the inlet element.

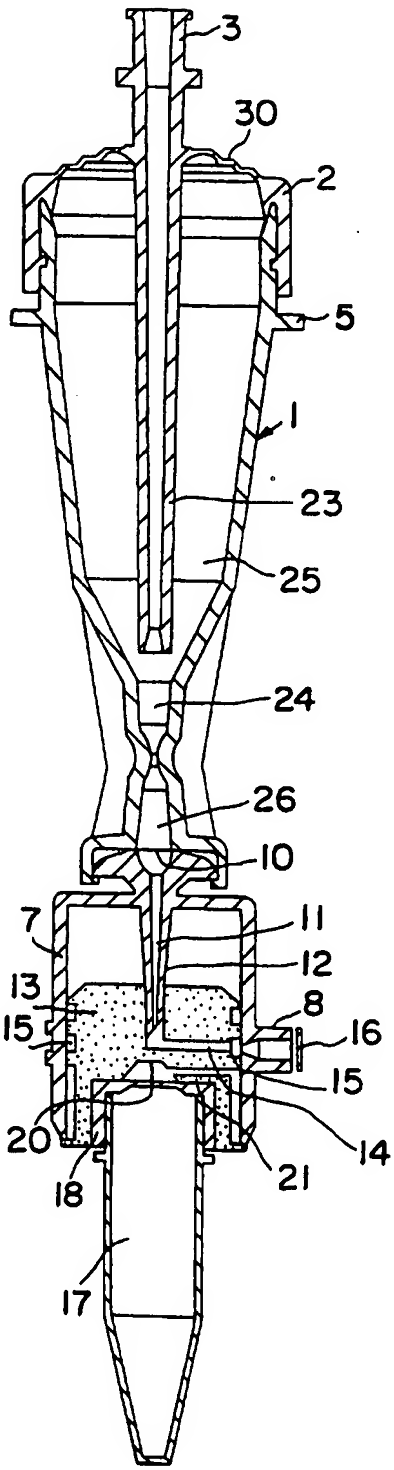
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FIG. 1

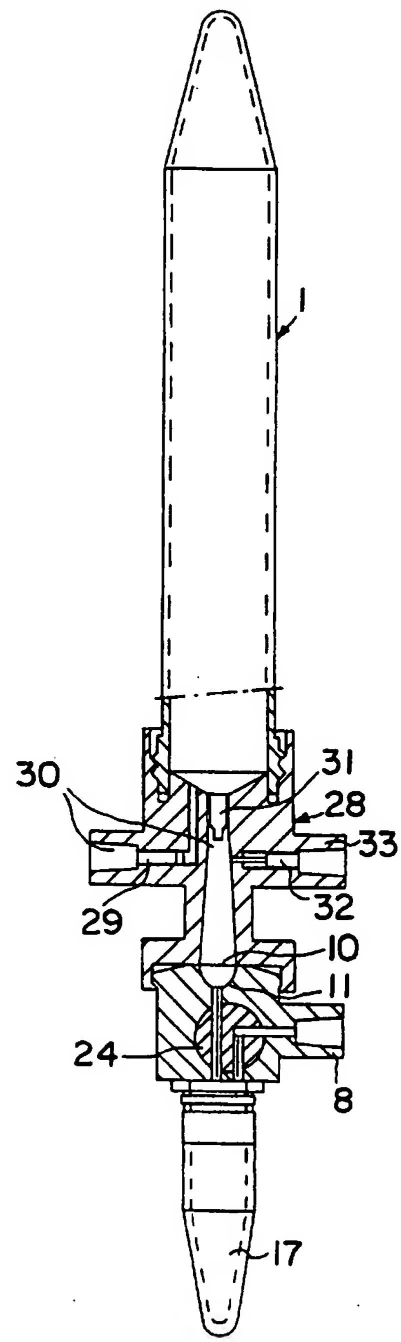
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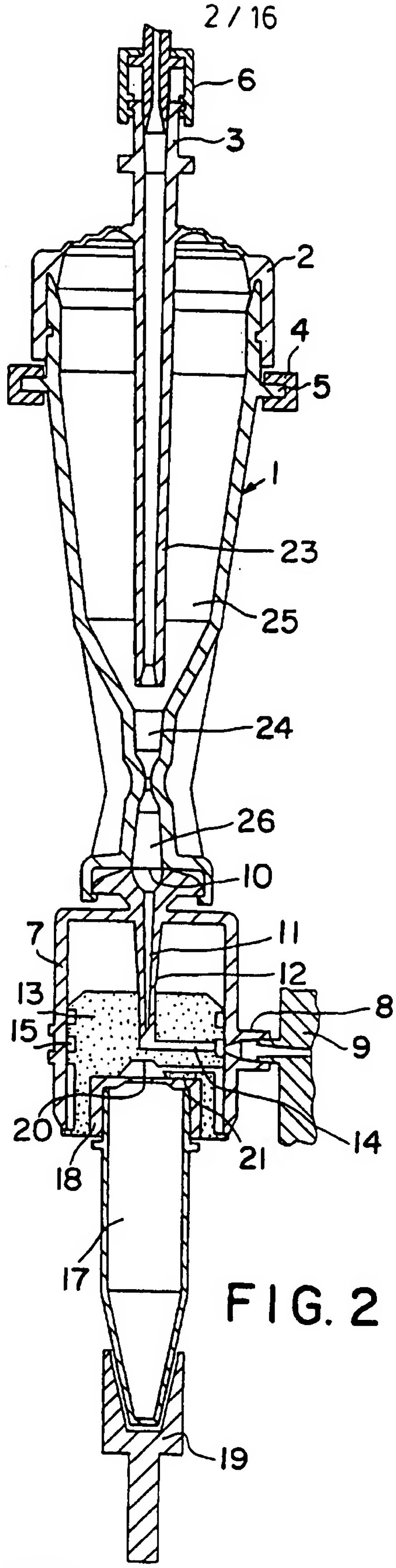
FIG. 7



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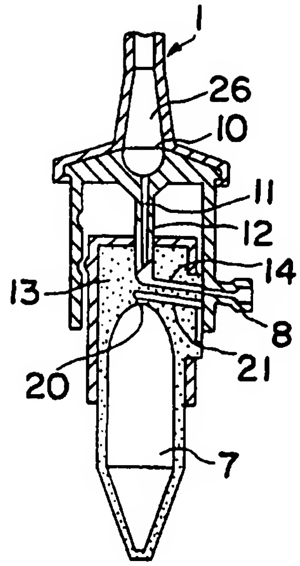

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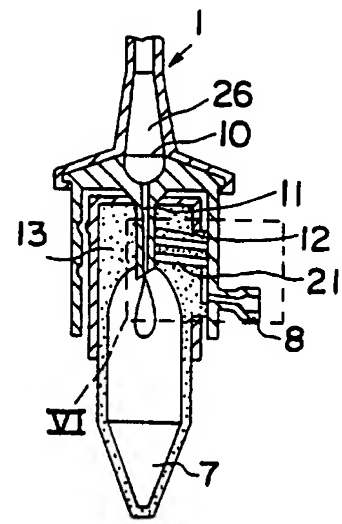

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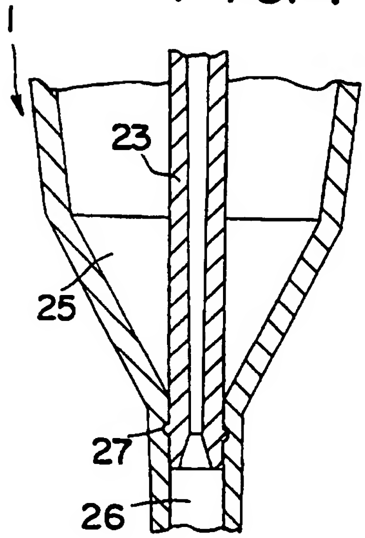

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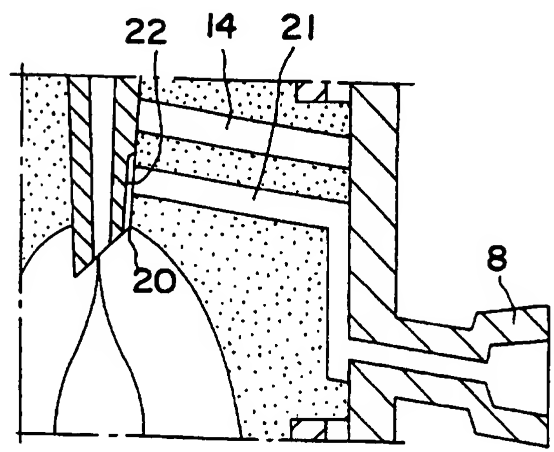

FIG. 6

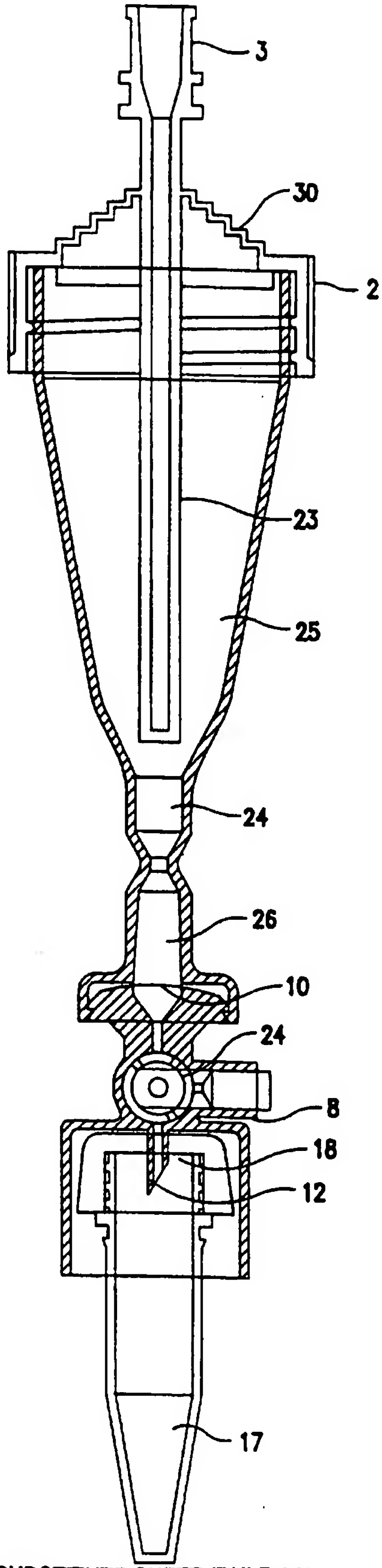

FIG. 8

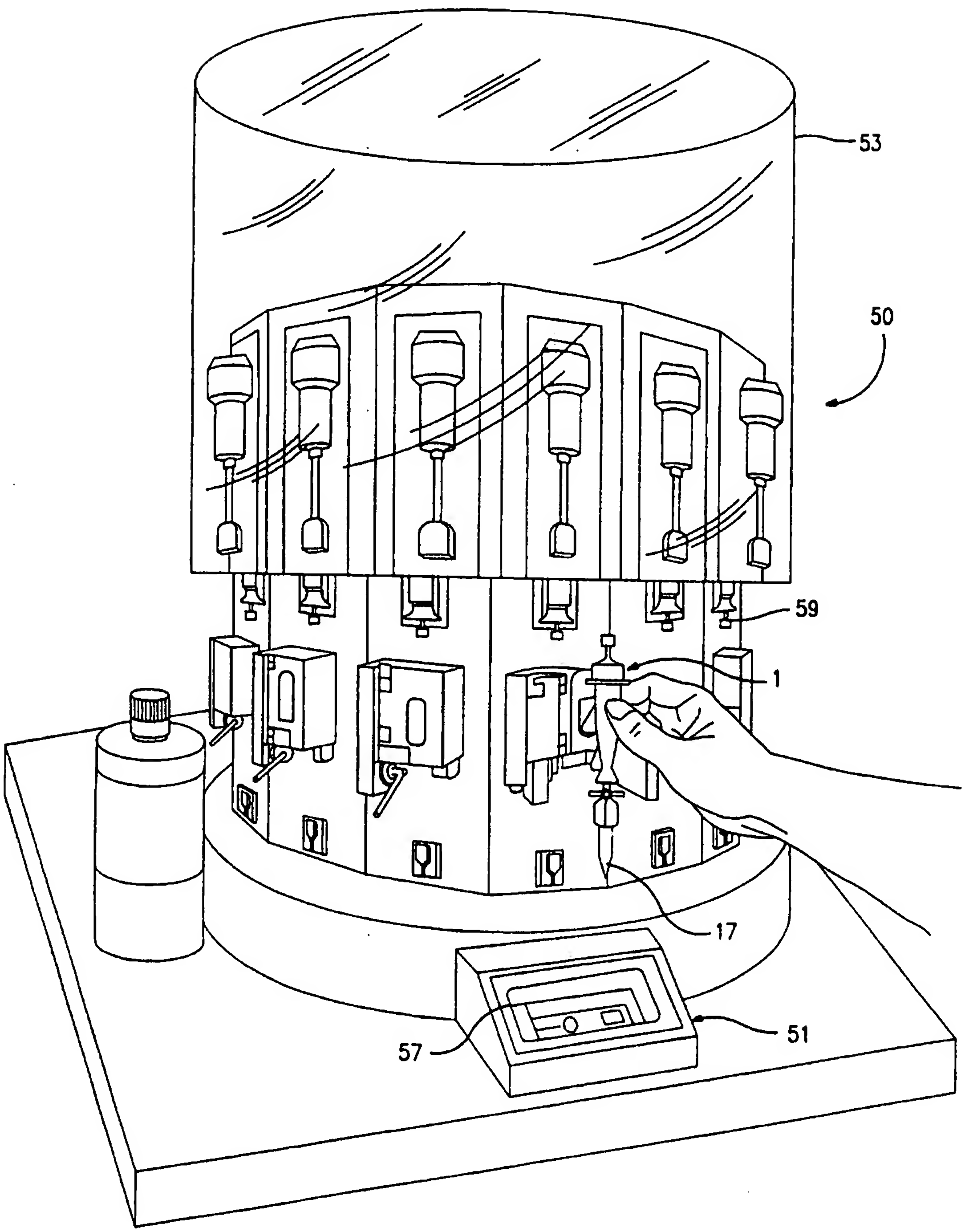

FIG. 9

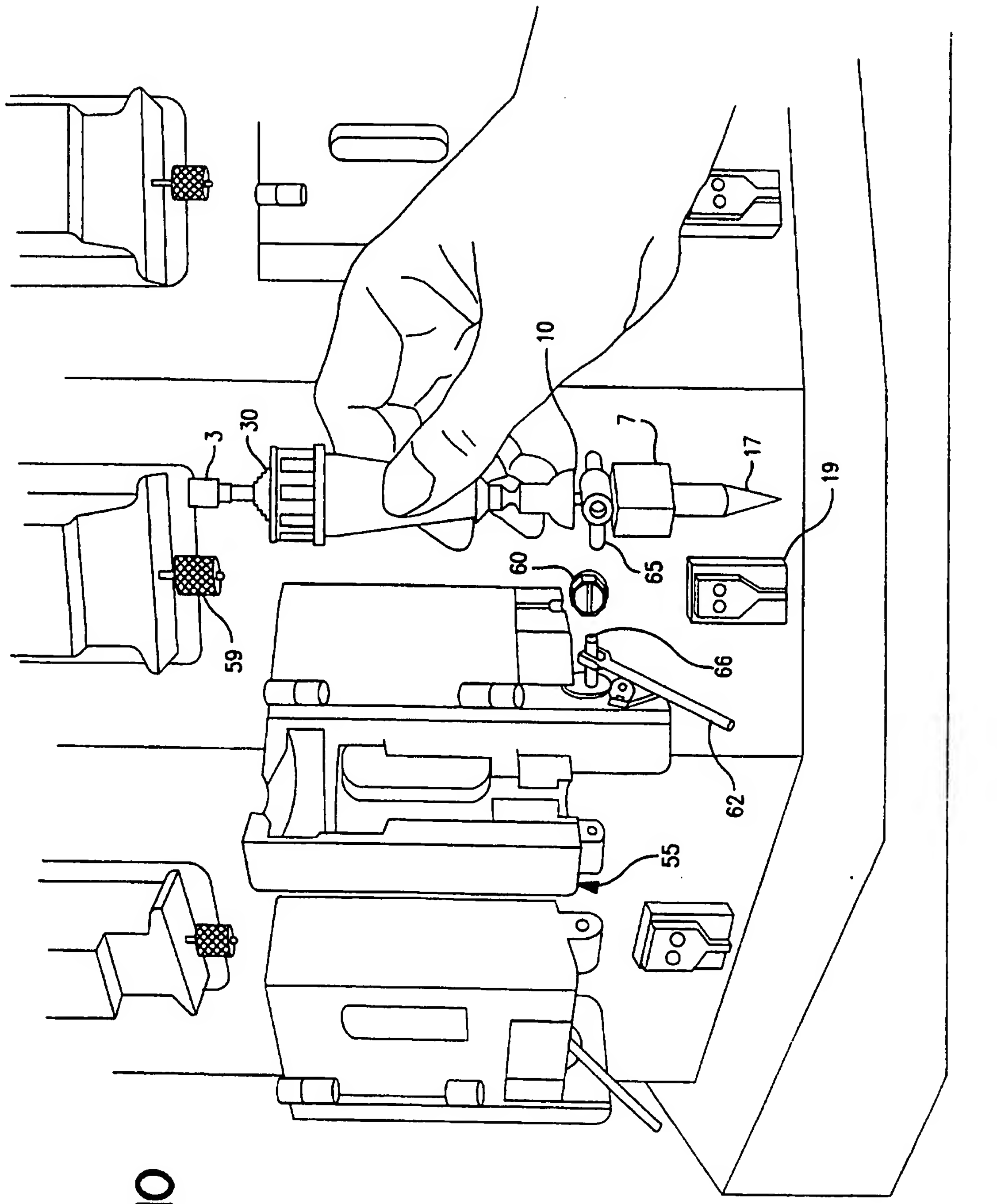

FIG. 10

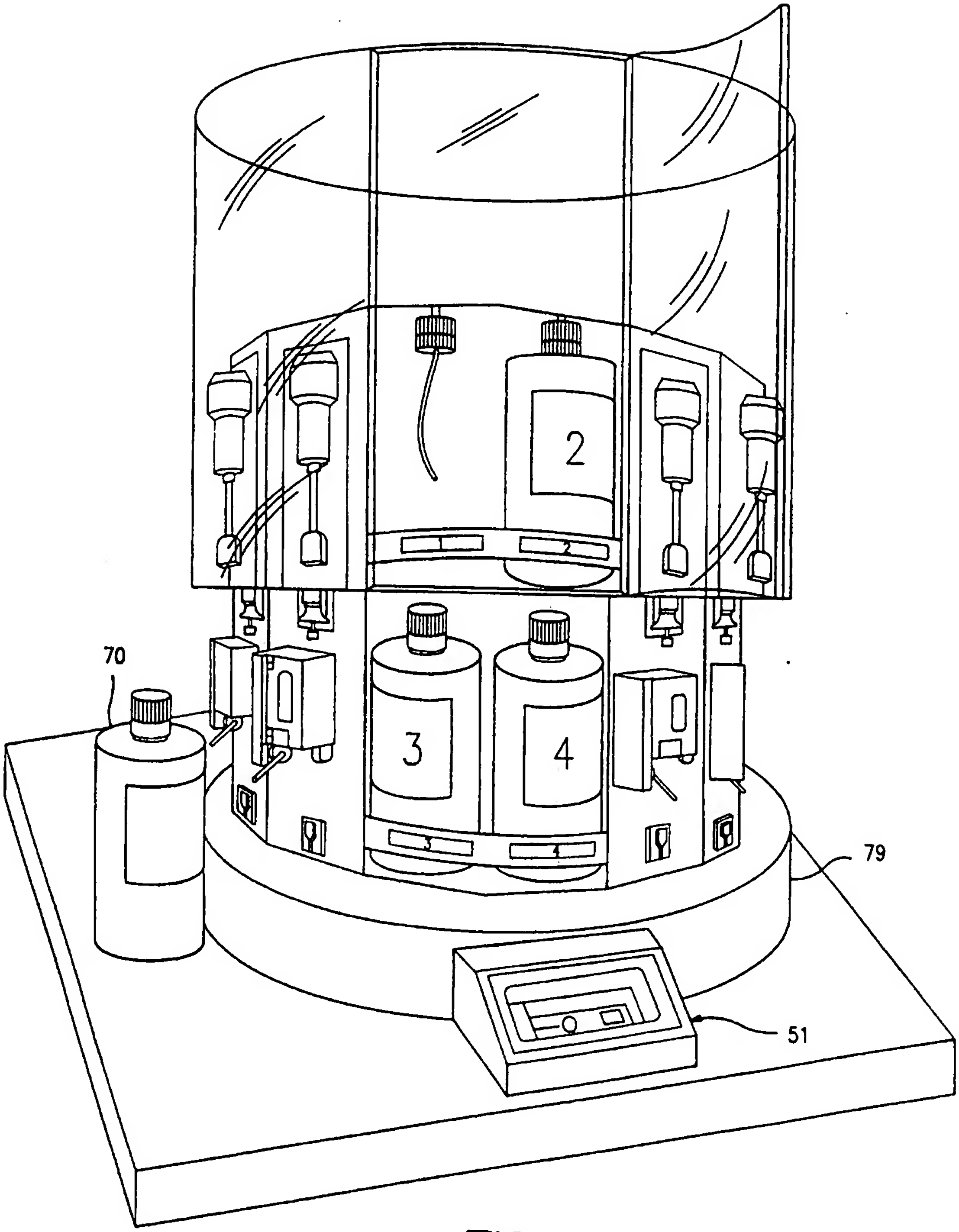

FIG. 11

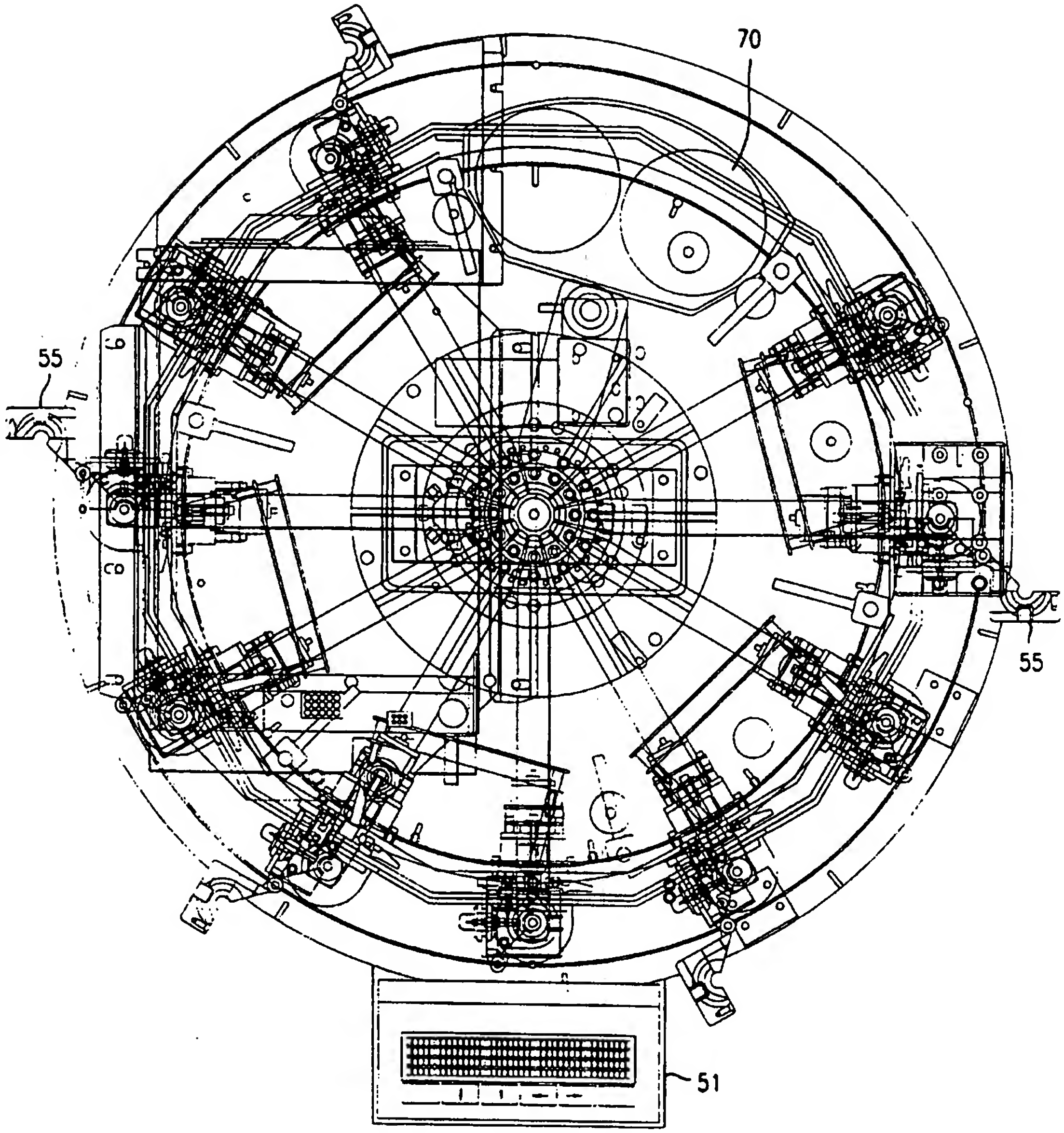

FIG. 12

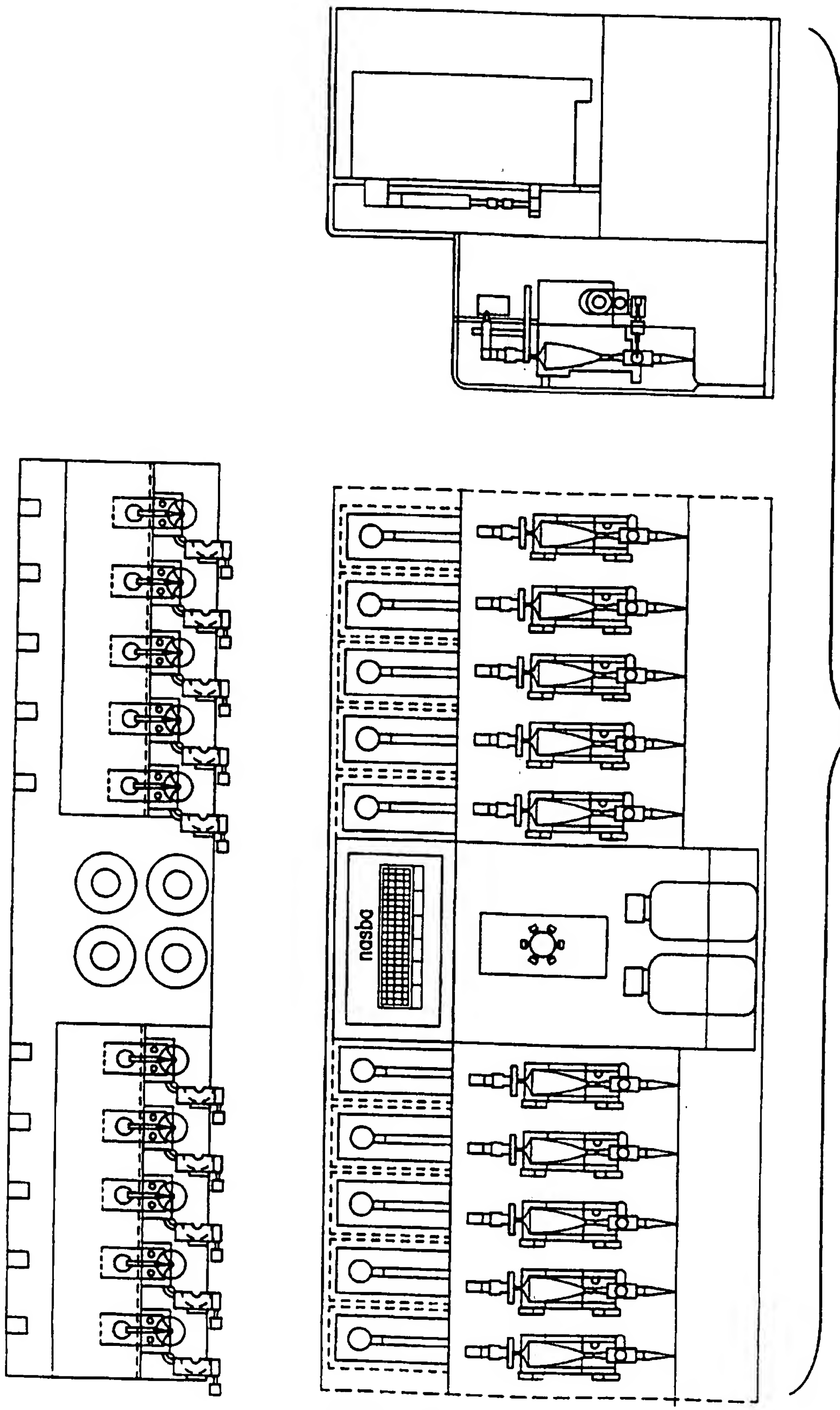

FIG. 13

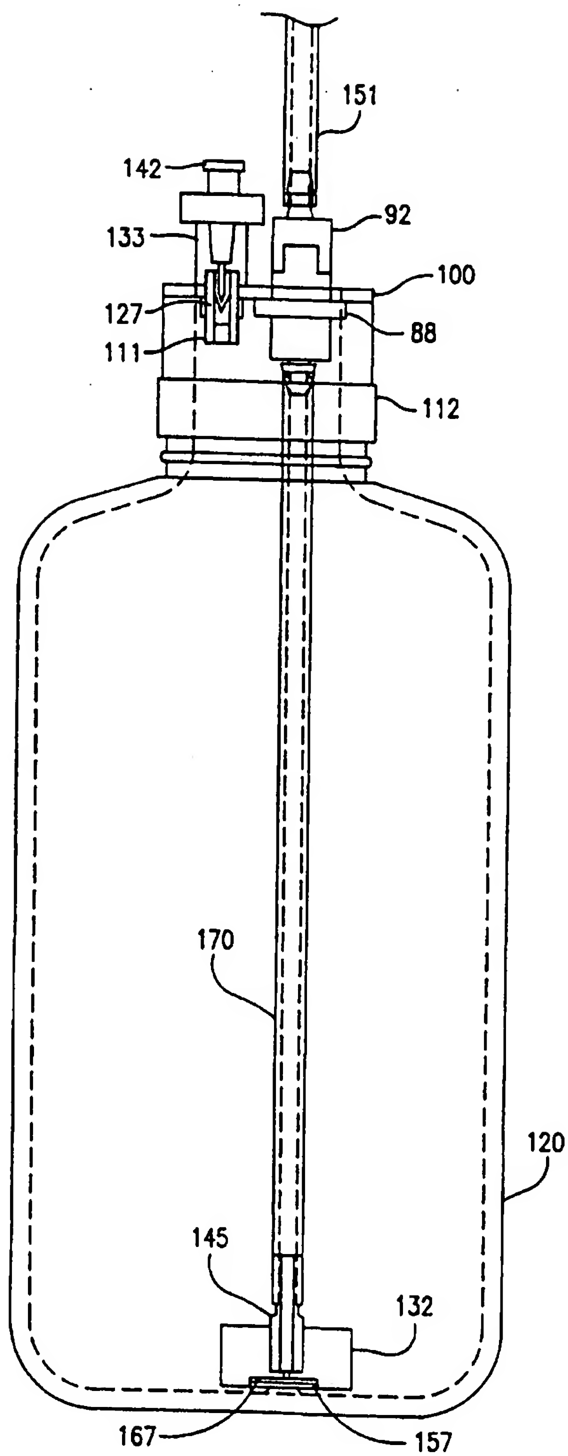

FIG. 14

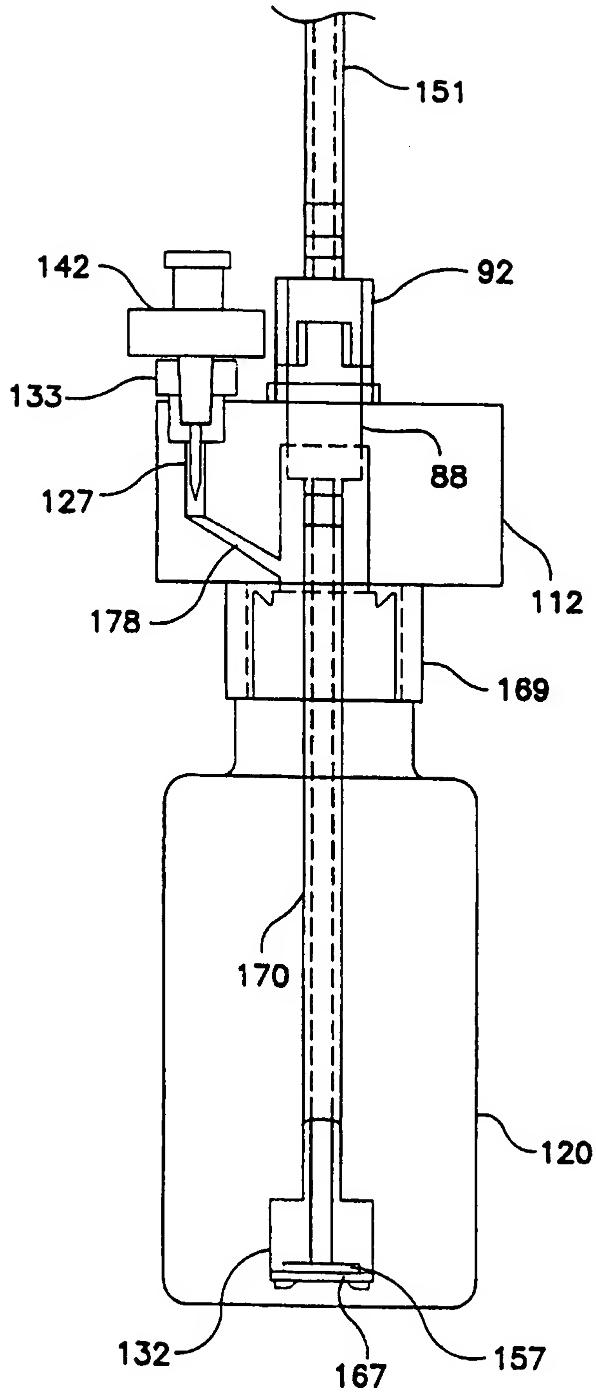
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FIG. 15

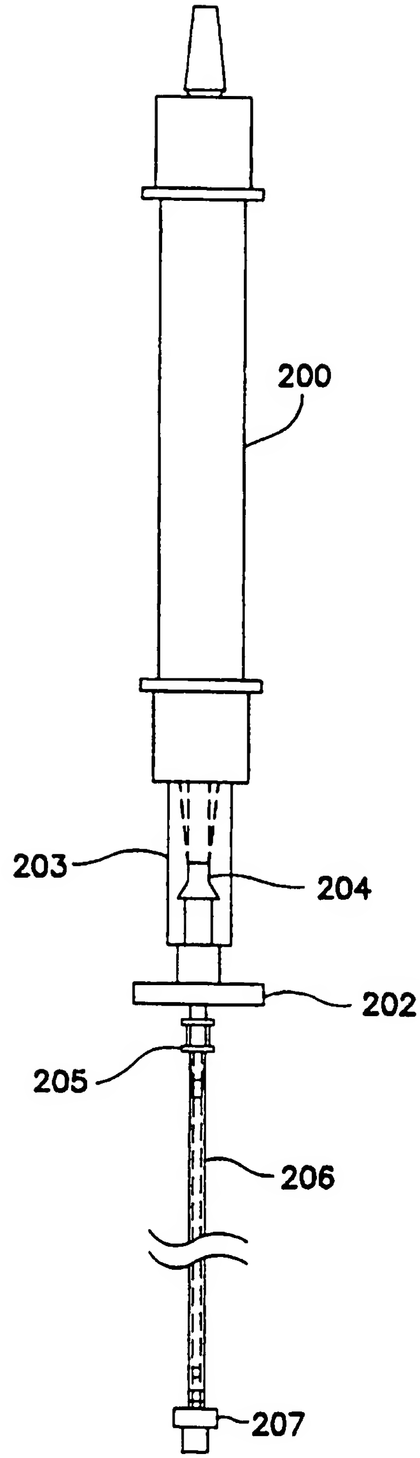
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FIG. 16

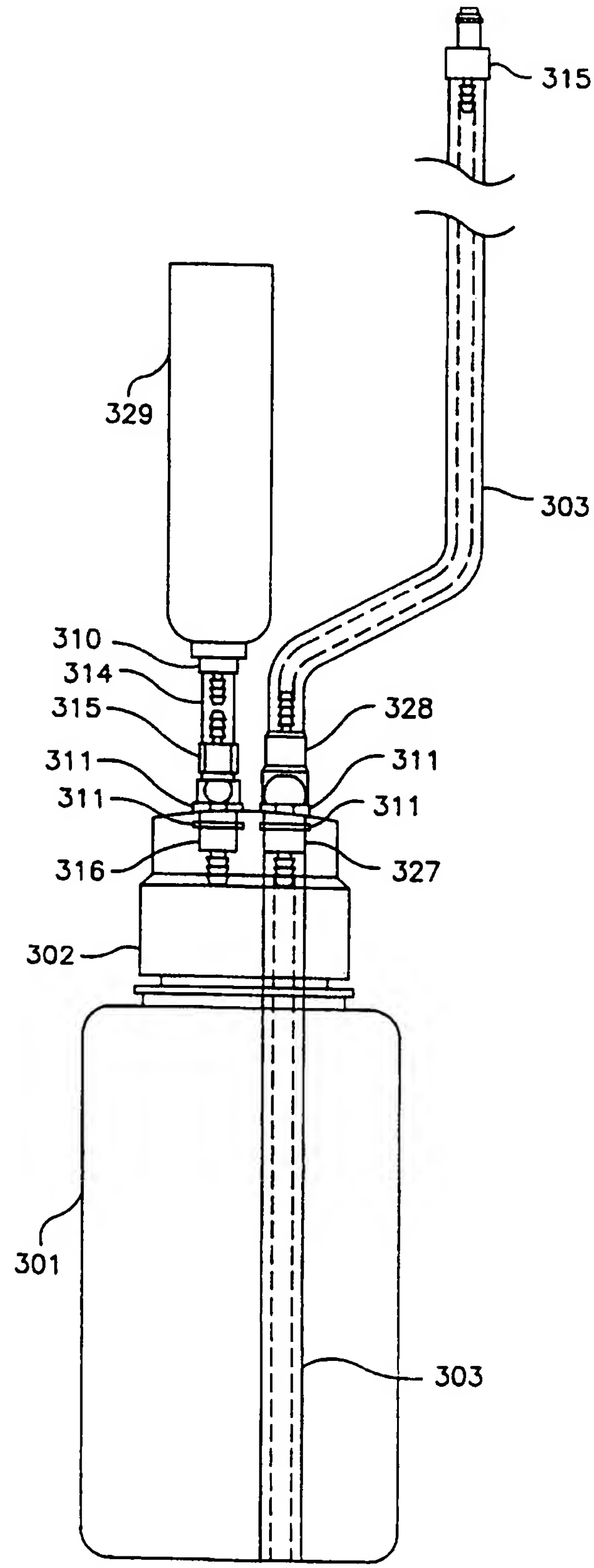

FIG. 17

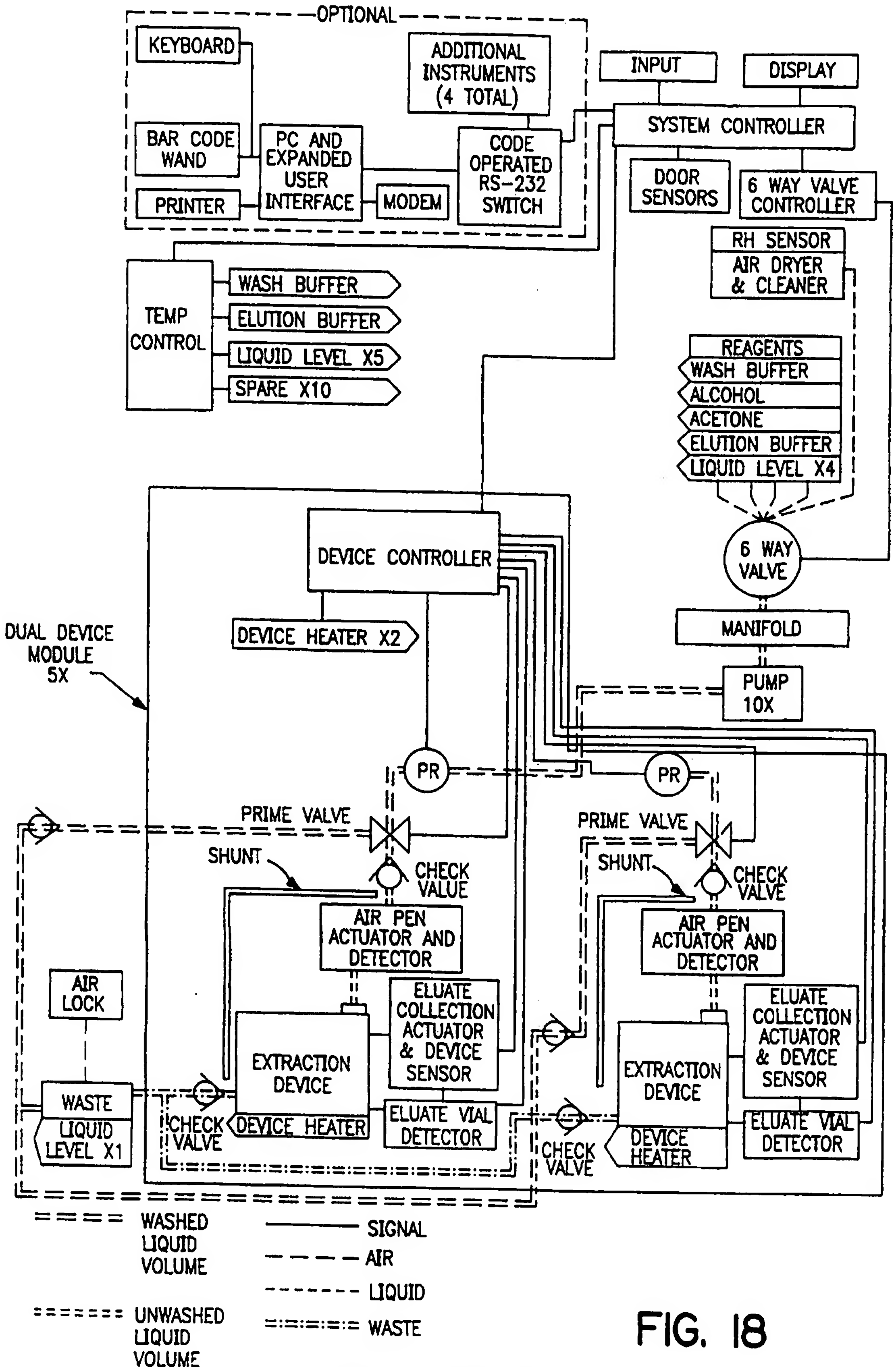
OPTIONAL
KEYBOARD
BAR CODE WAND
PRINTER
PC AND EXPANDED USER INTERFACE
MODEM
ADDITIONAL INSTRUMENTS (4 TOTAL)
CODE OPERATED RS-232 SWITCH
INPUT
DISPLAY
SYSTEM CONTROLLER
DOOR SENSORS
6 WAY VALVE CONTROLLER
RH SENSOR
AIR DRYER & CLEANER
TEMP CONTROL
WASH BUFFER
ELUTION BUFFER
LIQUID LEVEL X5
SPARE X10
REAGENTS
WASH BUFFER
ALCOHOL
ACETONE
ELUTION BUFFER
LIQUID LEVEL X4
6 WAY VALVE
MANIFOLD
PUMP 10X
DEVICE CONTROLLER
DEVICE HEATER X2
DUAL DEVICE MODULE 5X
PR
PRIME VALVE
SHUNT
CHECK VALUE
AIR PEN ACTUATOR AND DETECTOR
ELUATE COLLECTION ACTUATOR & DEVICE SENSOR
EXTRACTION DEVICE
AIR LOCK
WASTE
LIQUID LEVEL X1
CHECK VALVE
DEVICE HEATER
ELUATE VIAL DETECTOR
PR
PRIME VALVE
SHUNT
CHECK VALVE
AIR PEN ACTUATOR AND DETECTOR
ELUATE COLLECTION ACTUATOR & DEVICE SENSOR
EXTRACTION DEVICE
DEVICE HEATER
ELUATE VIAL DETECTOR
CHECK VALVE
WASHED LIQUID VOLUME
UNWASHED LIQUID VOLUME
SIGNAL
AIR
LIQUID
WASTE

FIG. 18

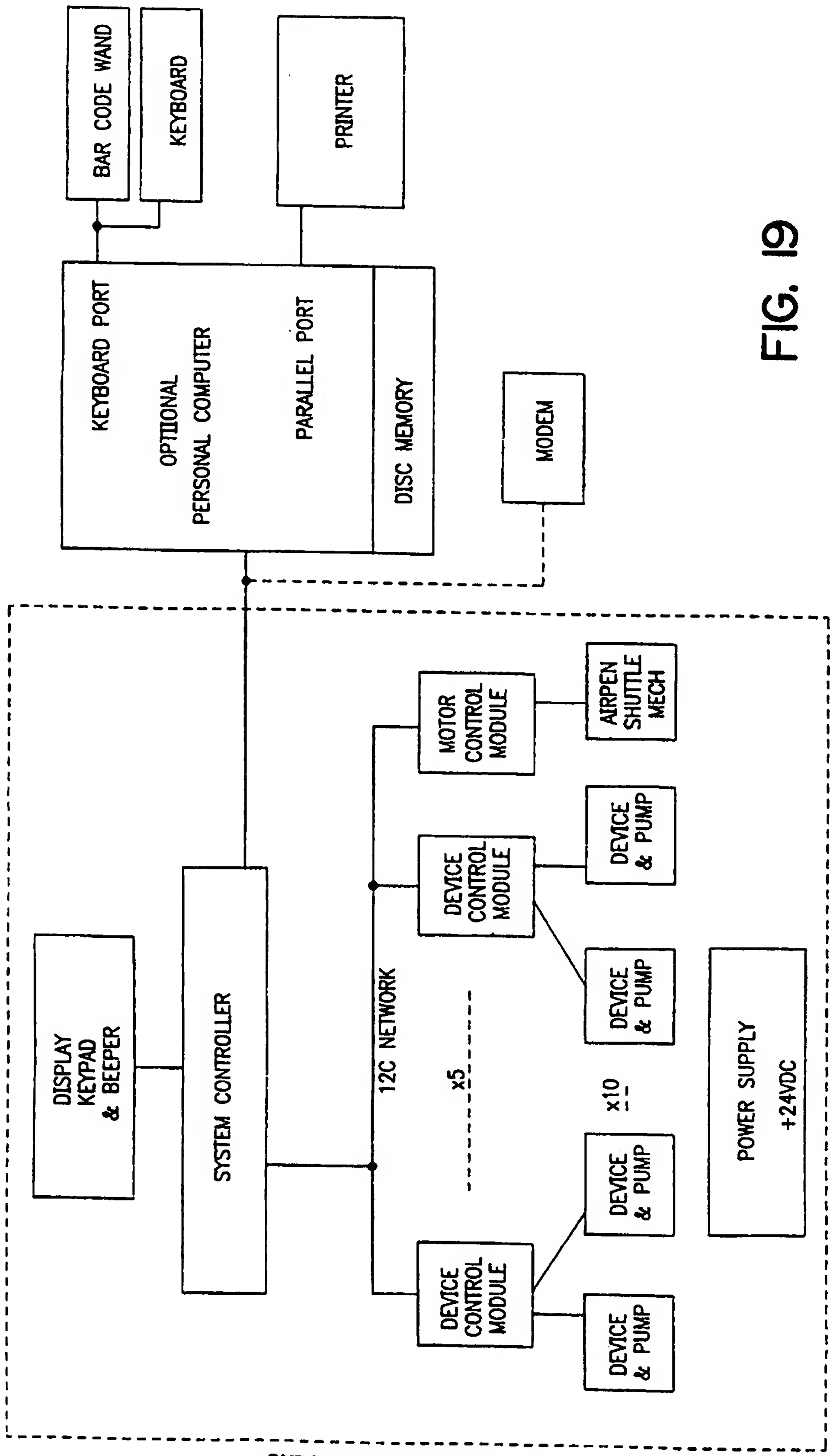
BAR CODE WAND
KEYBOARD
PRINTER
KEYBOARD PORT
OPTIIONAL PERSONAL COMPUTER
PARALLEL PORT
DISC MEMORY
MODEM
DISPLAY KEYPAD & BEEPER
SYSTEM CONTROLLER
12C NETWORK
x5
MOTOR CONTROL MODULE
AIRPEN SHUTTLE MECH
DEVICE CONTROL MODULE
DEVICE & PUMP
DEVICE & PUMP
x10
DEVICE CONTROL MODULE
DEVICE & PUMP
DEVICE & PUMP
POWER SUPPLY +24VDC

FIG. 19

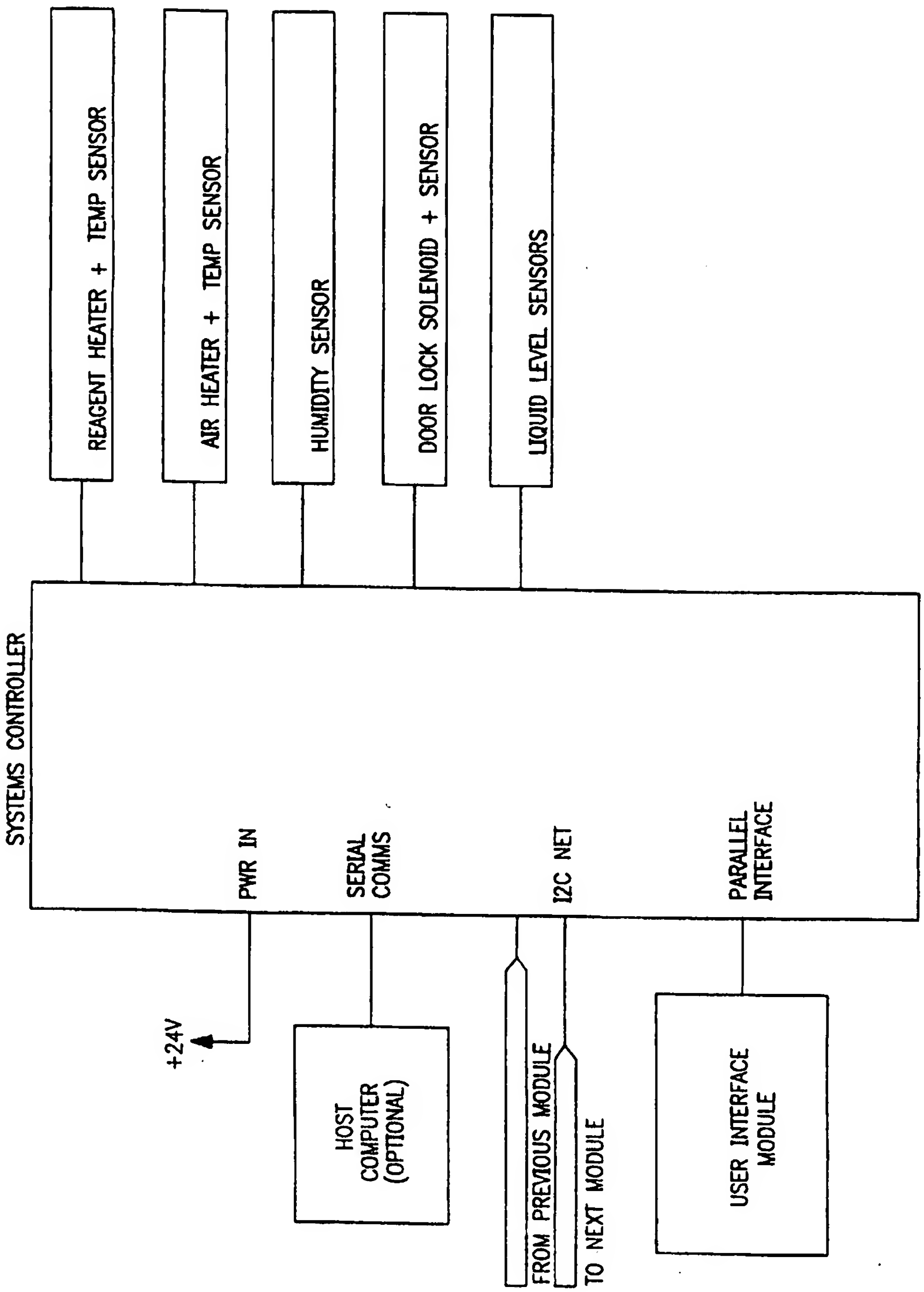
REAGENT HEATER + TEMP SENSOR
AIR HEATER + TEMP SENSOR
HUMIDITY SENSOR
DOOR LOCK SOLENOID + SENSOR
LIQUID LEVEL SENSORS
SYSTEMS CONTROLLER
PWR IN
SERIAL COMMS
I2C NET
PARALLEL INTERFACE
+24V
HOST COMPUTER (OPTIONAL)
FROM PREVIOUS MODULE
TO NEXT MODULE
USER INTERFACE MODULE

FIG. 20

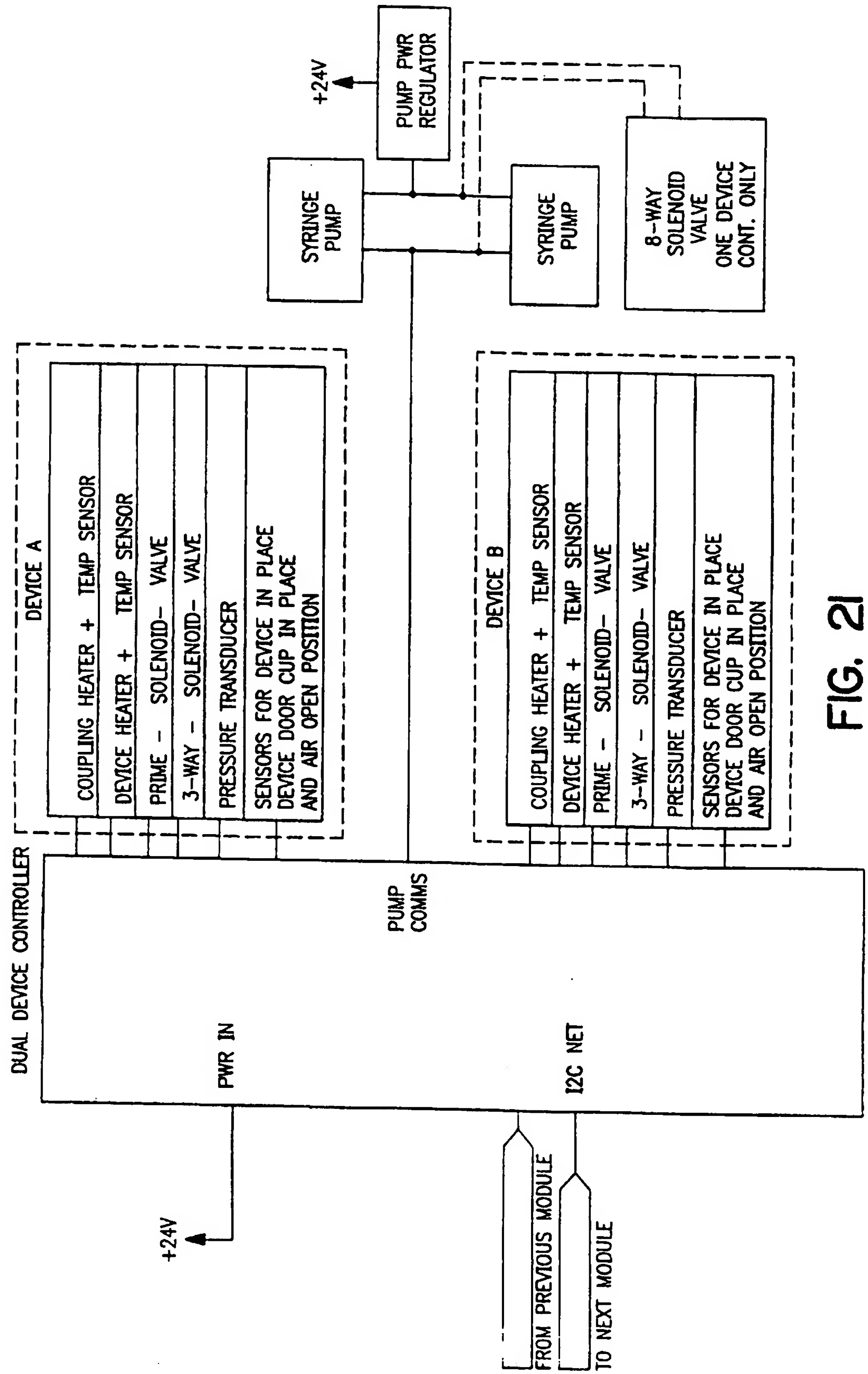
PUMP PWR REGULATOR
+24V
SYRINGE PUMP
SYRINGE PUMP
8-WAY SOLENOID VALVE ONE DEVICE CONT. ONLY
DEVICE A
COUPLING HEATER + TEMP SENSOR
DEVICE HEATER + TEMP SENSOR
PRIME - SOLENOID- VALVE
3-WAY - SOLENOID- VALVE
PRESSURE TRANSDUCER
SENSORS FOR DEVICE IN PLACE DEVICE DOOR CUP IN PLACE AND AIR OPEN POSITION
DEVICE B
COUPLING HEATER + TEMP SENSOR
DEVICE HEATER + TEMP SENSOR
PRIME - SOLENOID- VALVE
3-WAY - SOLENOID- VALVE
PRESSURE TRANSDUCER
SENSORS FOR DEVICE IN PLACE DEVICE DOOR CUP IN PLACE AND AIR OPEN POSITION
DUAL DEVICE CONTROLLER
PUMP COMMS
PWR IN
I2C NET
+24V
FROM PREVIOUS MODULE
TO NEXT MODULE

FIG. 2I

INTERNATIONAL SEARCH REPORT

Interr   al Application No
PCT/US 97/03533

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01D15/00 C07H1/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01D C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 443 734 A (FETNER) 22 August 1995 " IN TOTALITY " ---	1-5,7, 11,14, 21-38
P,A	WO 96 06850 A (AKZO NOBEL) 7 March 1996 see page 11-13; claims 1-15 ---	2-13, 36-46
A	US 5 234 809 A (BOOM) 10 August 1993 cited in the application ---	
A	US 5 114 858 A (WILLIAMS) 19 May 1992 see page 18-20; claim 3 ---	2,5
A	EP 0 487 028 A (SHIMADZU CORP.) 27 May 1992 -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 1997	08.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wendling, J-P

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern al Application No
PCT/US 97/03533

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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